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MULTIPLE MINUTE PULMONARY TUMORS RESEMBLING CHEMODECTOMAS

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In the microscopic study of lungs we have occasionally encountered distinctive perivascular cellular proliferations suggestive of chemodectomas in cytology and arrangement. Comparison with a rare instance of metastasizing carotid body tumor supports this interpretation. These pulmonary tumors are often multiple and are usually so small as to escape detection with the unaided eye. The cells occur in solid nests or "*Zellballen*" which are invariably related to small pulmonary veins. The consistency of this vascular relationship strengthens the concept of a special cellular origin. Although the precise nature of this origin is still not entirely certain, the cells appear to be neither endothelial nor related to the lining cells of air spaces. It is our aim to define this entity and to describe features that distinguish it from angioma and from the relatively common atypical epithelial proliferations of the lung.

Although there is abundant physiologic evidence of the existence of chemoreceptors in the lung, none have as yet been identified microscopically. Possibly the study of these tumors will provide a clue to the solution of this problem. We hope also that further information will become available on the nature and significance of these lesions as they become more generally recognized.

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Material and Methods

Except for one case the lesions were encountered in random blocks. Fixation was variously in Helly's fluid, Zenker-formol solution, or 10 per cent formalin. Except for those blocks which had been embedded in paraffin, most of the tissue had been preserved in formalin or alcohol for many months. Histologic sections were cut from paraffin at 6 μ. The stains included hematoxylin and eosin, Mallory's aniline blue, Masson-Goldner trichome, Verhoeff's elastic stain, the Gordon and Sweets or Laidlaw stain for reticulum, the Hotchkiss periodic acid-Schiff (PAS) with diastase control, the Prussian blue reaction for iron, the modified Giemsa method for chromaffin,[1] a diazo method for argentaffin cell granules, the Smith-Dietrich modification of Baker's acid-hematein method for selective staining of red corpuscles, and the Bodian stain for nerve fibers. Frozen sections were examined with the Sudan fat stain, Baker's acid-hematein method for phospholipids, Gomori's stain for alkaline phosphatase, and the Cajal silver impregnation for nerve fibers. Many of these methods were used despite the limitations imposed by prolonged fixation.

In case 18, where multiple small tumors were detected grossly, numerous blocks were embedded, and several were serially sectioned. In 2 lesions from this case, and in 2 of 15 lesions from another (case 2), complete sets of serial sections were available, and useful information regarding the vascular relationships was obtained. To investigate the vascular relationships, the outline of the lesion and its vessels was drawn on thin tracing paper with the aid of a camera lucida. The tracings were then superimposed in the correct order, transilluminated, and photographed,* whereupon the arterial supply was clearly demonstrated.

An attempt was made to inject the vessels in case 18. One lung was injected with vinyl plastic by a method previously described.[2] A low viscosity (7 per cent) green material was used for initial filling of the veins, and 7 per cent red plastic was employed to fill the arteries initially. Vessels approximating 50 μ in diameter and larger can be injected by this method. Subsequently, the more proximal vessels were filled with correspondingly colored 28 per cent plastic containing 10 per cent diatomaceous earth. Concentrated white plastic was used for the air passages. Another lobe was injected by Schlesinger's gelatin-KI-formol method,[3] with $BaSO_4$ for the veins, and with colloidal carbon (Stirling R. Carbon Black, G. L. Cabot, Inc., Boston, Massachusetts) to color black the material injected into the pulmonary arteries. Blocks several mm. thick were cleared by the method of Spalteholz for microscopic examination.

Observations

Prevalence

Only a crude estimate of the prevalence of these lesions can be given at this time. Twelve cases were encountered in a total of 3,635 necropsies (Table I, MGH series), but no systematic survey of the necropsy population was made, and the lesions were found in random sections, representing only very small samples of lung tissue. On the basis of these incomplete data it seems clear that the actual frequency of these lesions must be greater than one in every 300 necropsies.

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Clinical Correlations

A few data regarding the 19 patients under study are recorded in Table I. Fourteen were women. All of the patients were over 40 years of age, and most were over 60, but this may only reflect the age characteristics of our necropsy populations. It is not surprising, therefore, that pulmonary emphysema was noted in 4 cases and pulmonary fibrosis in one. One patient had a long history of asthma (case 18). Congestive heart failure was the most common major associated condition, and most of the lungs under study were congested and edematous. To consider a possible relationship of these lesions to pulmonary venous hypertension, lung sections from 40 patients with severe mitral stenosis were examined. In none was a lesion found. Four of the patients suffered from diabetes mellitus. Six had extrapulmonary neoplasms, but only two of these, both cerebral glioblastomas, were alike, and none had metastasized to the lung. Moreover, none of the primary tumors resembled the pulmonary lesion. This review, then, yields little of note, except possibly significant data regarding age and sex incidence.

Gross Features

The lesions were recognized grossly at first inspection only in case 18, whereupon they were identified in frozen section. Literally hundreds of these structures were present in all lobes, predominantly in the peripheral portions, and many were immediately subpleural. Many lay at the septal insertions upon the pleura where characteristic concentrations of black pigment were distributed over them. This position is to be expected from the relationships of the lesions to the veins. They were gray-pink, translucent, firm elevations, rounded but somewhat irregular (Figs. 1 and 2). They bulged above the cut surface, were unencapsulated, and showed no evidence of hemorrhage or necrosis. They were sometimes clearly seen to be centered upon venules (Fig. 2). No lesions were seen in the lymph nodes. In no instance were the tumors related to the scars present in some of the lungs.

Microscopic Features

Relationship to Veins. Among the most interesting and characteristic features of these tumors was their interstitial location and their relationship to blood vessels. They were always centered upon pulmonary venules (Figs. 3, 4, 5 and 11) and consequently upon the septums, especially where the latter inserted upon the pleura. The larger of these venules had diameters in the range of 200 μ. The rounded polyhedral or spindle-shaped cells were arranged in nests and masses delimited by bands of

TABLE I

PULMONARY CHEMODECTOMA. DATA REGARDING PATIENTS AND ASSOCIATED PATHOLOGIC DISORDERS

Case no.	Necropsy no.	Age	Sex	Major necropsy observations	Pulmonary abnormality	Chemodectomas found
1	MGH 18794	62	F	Glioblastoma multiforme	Pulmonary edema	2
2	MGH 19261	65	F	Cystadenocarcinoma of ovary with carcinomatosis peritonei; rheumatic heart disease, healed aortic and mitral valvulitis	Chronic passive congestion of lungs; pulmonary emboli, multiple, recent; pulmonary atelectasis, right lower lobe	15
3	MGH 19276	62	F	Ruptured saccular aneurysm, right internal carotid artery, subarachnoid hemorrhage	Pulmonary congestion and edema	4
4	MGH 19392	72	M	Coronary heart disease, recent and old myocardial infarcts	Pulmonary congestion and edema	1
5	MGH 20419	49	M	Coronary heart disease, recent and old myocardial infarcts	Pulmonary atelectasis, focal, slight; pulmonary fibrosis, diffuse, moderate	3
6	MGH 20420	76	F	Hemochromatosis, generalized	None (lungs not affected by hemochromatosis)	7
7	MGH 20947	72	M	Adenocarcinoma of rectum	Pulmonary emphysema, moderate; pulmonary emboli, multiple, recent	1
8	MGH 21179	55	M	Squamous cell carcinoma, trachea, metastases to heart and pericardium; arteriosclerotic heart disease, old myocardial infarct and mural thrombi in both atria	Pulmonary congestion and edema; pulmonary emboli, multiple, recent	1
9	MGH 21432	78	F	Carcinoma of breast, metastases to liver	None; (no metastases found)	1
10	MGH 22192	78	F	Arteriosclerotic heart disease	None	1

11	MGH 22198	56	F	Diabetic coma	None	1
12	MGH 22429	68	F	Hypertension; diabetes mellitus; arteriosclerotic heart disease; glioblastoma, left cerebral	Pulmonary congestion and edema	2
13	NHH 253	91	F	Bronchopneumonia; cerebral infarct; verrucous mitral endocarditis	Pulmonary emphysema; chronic bronchitis	2
14	NHH 8543	43	F	(Congestive heart failure). Hypertensive heart disease	Pulmonary congestion and edema	2
15	NHH 11572	73	F	Carcinoma of pancreas, metastases to liver	Pulmonary emphysema; pulmonary congestion. (No pulmonary metastases found)	1
16	NHH 13538	41	M	Arteriosclerotic heart disease	Pulmonary congestion and edema	1
17	NHH 42 *	67	F	Cerebral hemorrhage	Pulmonary emphysema; left apical scar; calcified nodule, left lower lobe; pulmonary infarcts, focal, recent	1
18	NHH 12459	57	F	(Asthma)	Pulmonary congestion and edema	20 +
19	NHH 14135	86	F	Diabetes; epilepsy; arteriosclerotic heart disease	Pulmonary congestion and edema	1

* Outside.

collagen and elastic tissue of the vascular adventitia. These relationships were best brought out in Verhoeff stains of one of the larger lesions (Fig. 5). As one end a vein 80 μ in diameter was met in oblique section. The elastica extended about ramifying, sinuous, interanastomosing tributaries of this vessel, which formed vascular capsules not only of venules but also of capillaries for the *Zellballen* (Figs. 5 to 8). At some points, smaller branches of these venules broke through the elastic barrier, and then, free of elastica, came into more intimate relationship with the nests of tumor cells to which they yielded small numbers of capillaries (Fig. 8). The individual cell clumps were not concentrically situated about these vessels; rather, the latter ramified irregularly among them. This was best demonstrated with reticulum stains (Figs. 9 and 26). Actually the vascularity of the *Zellballen,* as studied with Baker's acid hematein method, was not great. At no point were the blood-containing spaces lined by tumor cells, but there was a definite endothelial lining, and connective tissue stains often demonstrated an additional thin subendothelial lamina of a substance staining like collagen.

Air spaces were inevitably approached as the tumor cells extended centrifugally in company with venules, but in no instance was the wall of a bronchiole invaded. The purely interstitial position of the tumor cells persisted even as the interalveolar septums were penetrated. Remarkably, a delicate barrier of capillaries supported by connective tissue always separated the cell nests from the air spaces (Fig. 10). This relationship was maintained even in the case of the most minute tumors that consisted merely of a few *Zellballen* intruding into interalveolar septums (Fig. 11). In some places distal air spaces had actually become surrounded and compressed by the burgeoning masses of tumor cells, but they could be identified still by persisting pigmented mononuclear cells within their compressed lumens (Fig. 12).

Arterial Supply. The arterial supply to these tumors was remarkably scanty and was difficult to trace. In 3 of the lesions sectioned serially, a few small pulmonary arterioles were found to enter the nodules and to distribute themselves irregularly into capillaries among the tumor cells (Figs. 13 to 15). This was to be expected as invasion occurred into the walls of air spaces to which pulmonary arterioles were normally distributed, and can be interpreted simply as entrapment of arterioles. No evidence was found of a blood supply from bronchial arteries in the serial sections, but it is quite possible that tiny arterioles escaped detection. Possibly cells so intimately related to an anastomosing system of pulmonary venules and capillaries require no additional arterial supply. Certainly at least the former vessels contain highly oxygenated blood, the oxygen saturation of which can only be reduced by admixture of blood from pulmonary arterioles.

Injection Studies. Arteriovenous anastomoses, either in association with these lesions or elsewhere, were not discovered in the cleared pigmented gelatin injections, in the complete sets of serial sections, or in the vinylite casts, nor was a collateral arterial system of vessels injected in retrograde manner from the pulmonary arteries. Both the vinylite and the Schlesinger mass would be expected to traverse precapillary anastomoses exceeding a diameter of 50 μ. The minute vessels in the tumor nodules were not demonstrably injected. Intervenous anastomoses less than 1 mm. in diameter were seen in the septums and pleura, both in the vinylite casts and in the Schlesinger preparations (Fig. 16). These exist normally, but were perhaps increased in number in the present material.

Arrangement and Characteristics of the Cells. In consequence of the regular relationship to the vessels, the larger lesions presented an organized multilobulated appearance. The cells which composed these rounded *Zellballen* varied in size and shape. The larger cells were rounded or were polygonal and approximated 30 μ in diameter (Fig. 17). Their nuclei were usually ovoid, but often bent, were vesicular with fine chromatin granules, and were roughly twice the diameter of a red blood cell. Nucleoli were not prominent, and no mitotic figures were found. The cytoplasm was occasionally sharply defined but usually had indistinct borders. In some cells it was deeply staining and homogeneously eosinophilic without granular content, but often it was vacuolated or seemed entirely clear. The vacuoles were not stained with lipid or PAS stains. Other cells were quite elongated and appeared flattened or spindle-shaped, and, in some foci, even fibrillar (Figs. 18 and 19). The nuclei then became correspondingly drawn out. This appearance was entirely compatible with that of chemodectoma, as indicated particularly by comparison with a metastasizing carotid body tumor (Figs. 20 to 22). Stains for chromaffin substance, argentaffin granules, and alkaline phosphatase were all negative, but the significance of this is doubtful because of the long fixation of the tissue.

Within an occasional nest, the cells became flattened and whorled in more or less spiral fashion about a central, brightly eosinophilic body (Fig. 23). Elsewhere some were grouped about a much larger, granular, likewise intensely eosinophilic mass (Fig. 24). Clumps of fibrillar material with the staining properties of collagen were also associated with flattened cells in a few fields (Fig. 25).

Only in the lungs with chronic congestion, from patients with decompensated rheumatic heart disease and in one patient with hemochromatosis, was hemosiderin present in the endothelial cells lining blood vessels of the tumor, in the connective tissue, and rarely in some of the tumor cells. Small quantities of anthracotic pigment were also found in

the vicinity of the veins, as was to be expected, but never within the tumor cells (Fig. 26).

Diligent attempts were made to demonstrate nerves by the special stains previously described, but without success. All of the specimens, however, had been either previously embedded in paraffin or fixed for prolonged periods of time. A further search with supravital methods in fresh tissue is indicated.

Sclerosing changes, with obliteration of the vessels, atrophy of the cells, accumulation of hyaline deposit with the staining properties of collagen, and shrinkage of the lesion were observed in some of the multiple tumors in cases 7 and 8 and in one of two lesions in case 12 (Figs. 27 to 29). Such features are not uncommon in tumors of endocrine organs.

Discussion

Evidence for Probable Relationship to Chemoreceptors

The suggestion that certain rather common small tumors in the lungs represent chemodectomas is based on (a) cytologic characteristics; (b) arrangement of cells; (c) special relationship to vessels.

The cytologic characteristics were quite compatible with those of tumors of the carotid body and related structures as described by LeCompte [4,5] who has divided these tumors into 3 groups on the basis of differences in cell form: the "usual" type, the "adenoma-like" type, and the "angioma-like" type. For the most part, the present lesions were of the "angioma-like" type, with a few fields of the "usual" type. None were of the "adenoma-like" or second type described by LeCompte, except possibly for the large intrapulmonary tumor reported by Heppleston.[6] As in LeCompte's experience with carotid body tumors, mitotic figures were not found in any of the intrapulmonary lesions in the present series. It is interesting to note that hemosiderin was also present in the stroma of one of the carotid body tumors illustrated by LeCompte.[5]

The arrangement in *Zellballen* in the present group of tumors also was identical with that of the carotid body and its tumors, and this was especially well brought out by the reticulum stain (compare Figs. 9 and 26 of the present report with Figs. 2 and 8 of LeCompte [5]). In both instances the small vessels were predominantly in the connective tissue that lay among the *Zellballen,* while the latter were almost solid cellular structures. The relationship was similar to that which bayberries bear to a twig. The cells of the individual *Zellballen* themselves were not concentrically related to blood vessels.

The relationship of the pulmonary chemodectomas to veins rather than to arteries, as in the case of the carotid body tumors, has its analogue in the tumors of the glomus jugulare,[7,8] which are situated in the adventitia

of the jugular bulb. In the lung the strategic location of these tumors along the intervenous bridges provoked the speculation that they might arise from a normal organule sensitive to changes in the composition of blood after its equilibration with the alveolar gases. The multiplicity, wide distribution, and tiny size of these lesions would be consistent with their origin from such hypothetical receptors. We have maintained a watch for anything that might be interpreted as a normal chemoreceptor in the lung. Only once has this been rewarded in the discovery of a group of cells in the adventitia of a pulmonary vein (Figs. 30 and 31), associated with a small nerve and twigs of systemic arterioles. The vein was larger than any related to the minute tumors under discussion. Further observations will be necessary to establish the significance of such structures.

The very meager blood supply from arterioles suggests that these vessels merely became entrapped by the interstitial growth of the tumor cells into the walls of air spaces, and that they did not constitute an essential blood supply for the chemodectomas. It is of interest that large primary pulmonary neoplasms derive their arterial blood from the systemic circulation.[9]

The difficulty in finding nerves associated with the pulmonary lesions appeared to be no greater than with chemodectomas elsewhere.[5,8,10–12] Actually, staining blocks of fresh tissue may represent a better procedure which has not as yet been attempted with these tumors.

Convincing physiologic evidence of the existence of chemoreceptors in the lungs has been obtained.[13,14] For example, the vagus-mediated pulmonary depressor chemoreflex[14] results in reflex bradycardia and hypotension upon the introduction into the pulmonary artery of such substances as amidines, guanidines, isothioureas, and 5-hydroxytryptamine, while the vagus-mediated pulmonary respiratory chemoreflex[14] results in reflex apnea when such chemicals as amidine derivatives, certain antihistamines, and veratridine[15] are injected into the pulmonary artery. However, neither the location nor the anatomic structure of pulmonary chemoreceptors has been established with certainty. It will be of particular interest to compare the cellular architecture of the tumors under discussion with that of normal chemoreceptors in the lung when these are identified.

Possibly Related Tumors Previously Described

Under the title "A Carotid-Body-like Tumour in the Lung," Heppleston[6] described a subpleural lesion of slow growth discovered in a survey film in a 47-year-old male achondroplastic dwarf. He considered this to differ from bronchial adenoma of the carcinoid type, which it re-

sembled in part, on the basis of the arrangement of the cells and on the apparent lack of relationship to a bronchus at surgery. The operation consisted of "shelling out" the lesion. Relationships to vessels were not established. This tumor was reminiscent of the "adenoma-like" type of carotid body tumor as described by LeCompte,[5] and thus differed not only in size, but also in appearance of its component cells from those described in the present paper.

In their discussion of "hemangiopericytomas" of the soft tissues, McCormack and Gallivan [16] mentioned two minute tumors of the lung, at least one of which had a close resemblance to the lesion described in the present report.

Differentiation from Other Cellular Proliferations in the Lung

In view of the incidence of chemodectomas in the lung, it appears probable that they have been classified previously as some other type of benign cellular proliferation, from which, once recognized, they can be distinguished. Proliferations of this nature, which might be confused, include:

Hemangiopericytoma. The relationship of the "pericyte" of Zimmerman, considered by Stout [17–19] to be the source cell of the group of tumors designated as hemangiopericytoma, to the cells of the neuromyo-arterial glomus and to those of the presumed chemoreceptor organs is still not clearly established.[20–23] The ringlike or concentric arrangement of cells about vessels, characteristic of the glomus and of the hemangiopericytoma as originally described, is not present in the carotid body tumor or in the pulmonary tumors of the present series (compare Figs. 3 and 4 in McCormack and Gallivan's paper [16]). In the hemangiopericytoma and glomus tumor the characteristic reticular pattern, with delicate fibers enclosing individual cells, is totally different from the pattern of nests of cells separated by reticulin that is so typical of the carotid body tumor and of the present lesions. The absence of any demonstrable precapillary arteriovenous anastomoses in the pulmonary tumors, plus the absence of the neuroreticular stroma and the definitive vascular arrangement characteristic of the neuromyo-arterial glomus, all indicate that the pulmonary lesion is not a true glomus structure.

Angioma. The question of the angiomatous or endotheliomatous nature of the pulmonary chemodectomas is at once resolved by the fact that the tumor cells do not line vessels and are always separated from vascular lumens by distinct endothelial, and frequently hyaline, barriers. Moreover, miliary angiomas or arteriovenous fistulas,[24] and the sclerosing hemangiomas [25] have quite a distinctive fine structure.

Atypical Proliferation of Epithelium. Of the various types of atypical

proliferation in the lung, the chemodectomas are most likely to be confused with peripheral bronchial adenomas,[26,27] or with the less well organized, but similar, carcinoid-like collections of cells that may even bear some resemblance to oat cell tumors. Whitwell's term "tumourlets"[28] for the latter has received wide usage, although they were known long before his report in 1955.[29–31] "Tumourlets" of this type occur with considerable frequency in organizing pulmonary disease, especially bronchiectasis. The distinction from chemodectoma is based first and foremost on the relationship of the epithelial proliferations to air spaces (Fig. 32) which they line and fill. They are not interstitial, and a capillary barrier does not intervene between the cells and the lumens of the air spaces as with the chemodectomas.

In the peripheral bronchial adenomas of the carcinoid type, the arrangement is more that of interlacing elongated cell cords with a spoke-like arrangement relative to larger vascular channels, unlike that of the *Zellballen.*

The Plexiform or Angiomatoid Lesions of Pulmonary Hypertension. In pulmonary hypertension, whether "primary,"[32,33] or associated with large intracardiac left to right shunts,[34–39] or associated with such acquired conditions as schistosomiasis,[40,41] remarkable complex vascular masses interrupt and in part surround pulmonary arterioles. Although these were at one time thought to be of congenital origin and to represent arteriovenous fistulas, it is now clear from their occurrence in acquired as well as in other forms of pulmonary hypertension that they probably result from damage to pulmonary arterioles, associated with the high blood pressure itself. One interpretation is that they represent exuberant granulation tissue. This is fed in part by bronchial arteries, in process of organizing the fibrin deposited upon the remnants of damaged pulmonary arterioles.[32,33,42] These structures, in contrast with chemodectomas, are related to arteries rather than veins, they possess the angiomatoid appearance suggested by their name, and they are at least in large part intravascular in location (Figs. 33 and 34).

Paraganglia. With the available tissue, attempts to demonstrate chromaffin or argentaffin granules in the cells of these lesions were inconclusive. Although no chromaffin tissue has been described in the lung, we cannot with certainty exclude the possibility that these tumors might be derived from some type of chromaffinic paraganglionic structure. The relationship, if any, between chromaffin tissue and chemoreceptor organs (nonchromaffin paraganglia) has long been a source of confusion, and two recent publications are pertinent. Utilizing a meticulous technique, Lever, Lewis and Boyd[43] have demonstrated in carotid body cells the presence of chromaffinic, argentaffinic cytoplasmic granules which are re-

leased in response to reserpine and anoxia. Comline and Silver[44] have demonstrated the secretion of noradrenaline by the denervated adrenal of fetal sheep in response to anoxia. They have shown that this ability of adrenal medulla to respond directly to an anoxic stimulus is lost later in fetal life. These studies suggest the possibility of an intimate fundamental relationship between these two types of tissue.

Metastatic Neoplasm. Metastatic tumor in the lung can be distinguished easily from the chemodectomas. Metastatic neoplastic cells have little or no relationship to veins and often tend to invade the air spaces. The presence of mitotic figures and special cell characteristics should also help to identify metastatic lesions.

Summary and Conclusions

Minute, occasionally multiple tumors invariably related to anastomosing pulmonary venules were encountered in the lungs of 19 individuals. The tumors were composed of cell nests (*Zellballen*) with structural and cytologic features closely similar to those of chemodectomas. The patients were predominantly women more than 40 years of age. The tumors were not associated with scarring or with any specific pulmonary disease or physiologic disturbance. They were always interstitial and were separated from the lumens of distal air spaces by a definite barrier of capillaries. In these features they differed from the proliferations of blood vessels, arteriovenous communications, and the various types of atypical epithelial proliferation in the lung.

Although there is abundant physiologic evidence of the existence of chemoreceptors in the lung, their anatomic structure is not known. The minute tumors described may offer a clue to the solution of this problem.

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[*Illustrations follow*]

Legends for Figures

Except where indicated, photographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. Pulmonary chemodectoma. Case 18. Gross appearance, pleural aspect. Five nodules are seen (arrows); the largest has a greatest diameter of 3 mm. Several appear to lie astride septums as the latter attach to the pleura, and these are mottled with anthracotic pigment.

Fig. 2. Case 18. Cut surface of one lesion. The tissue bulges when incised. The nodule is centered upon a small vein (arrow) met in section above it, and which protrudes from it below like the stalk of a cherry. Millimeter scale at left.

Fig. 3. Case 2. Central position of a venule within the tumor. The vessel has a diameter of 180 μ. The lesion lies within the adventitia and septum that encloses the vein and extends peripherally around its tributaries. At the margin, as the walls of air spaces are invaded, the tumor cells maintain their interstitial position. × 60.

Fig. 4. Same lesion shown in Figure 3 at another level of section. The relationship to the vein and its tributaries is maintained. × 60.

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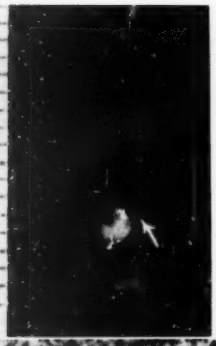

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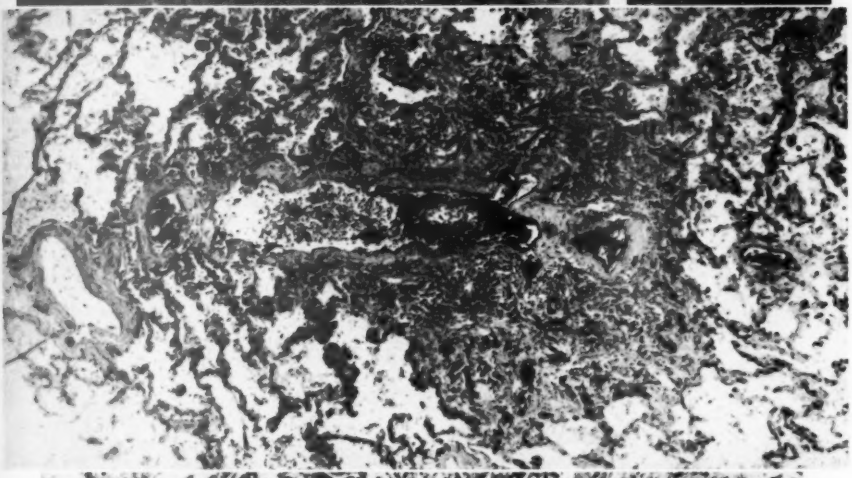

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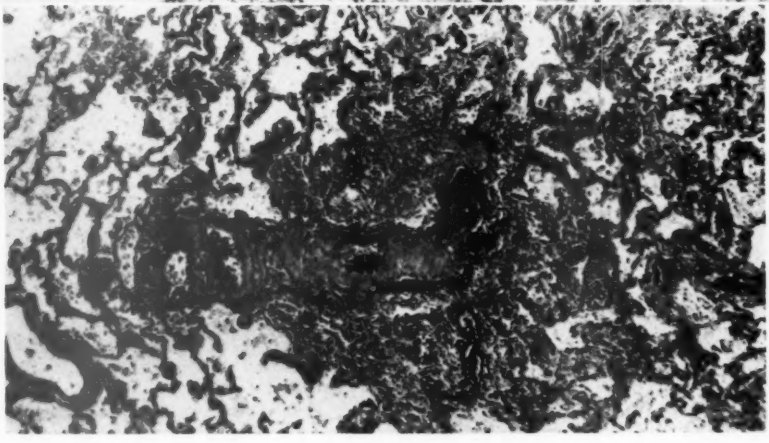

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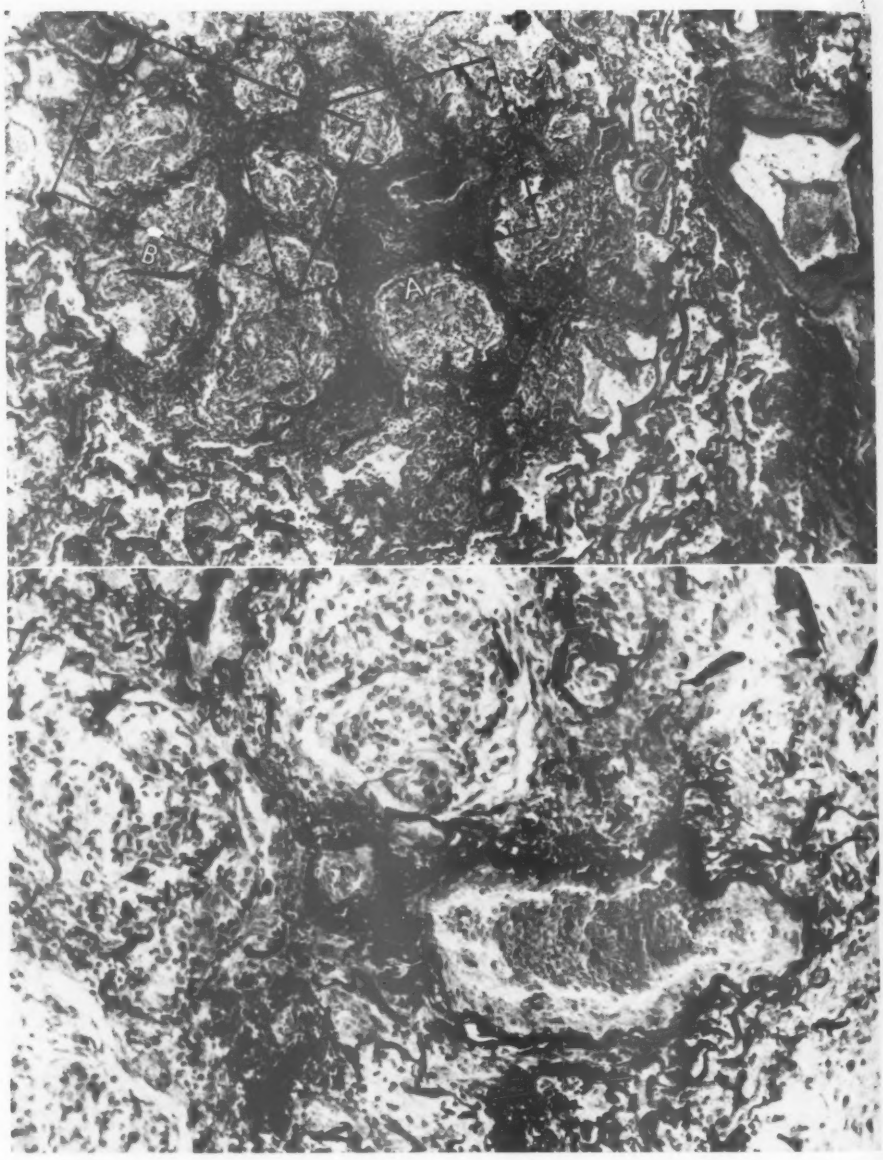

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FIG. 5. Case 2. A Verhoeff elastic stain of an entire nodule. The elastica of the septum and of the veins contained therein outlines distinct *Zellballen* for which the vessels provide vascular capsules. × 60. Field A is shown at higher magnification in Figure 6, and Field B in Figures 7 and 8.

FIG. 6. Field A of Figure 5 at higher magnification. Smaller veins are outlined by the elastica in the capsules of the *Zellballen*. Verhoeff elastic tissue stain. × 200.

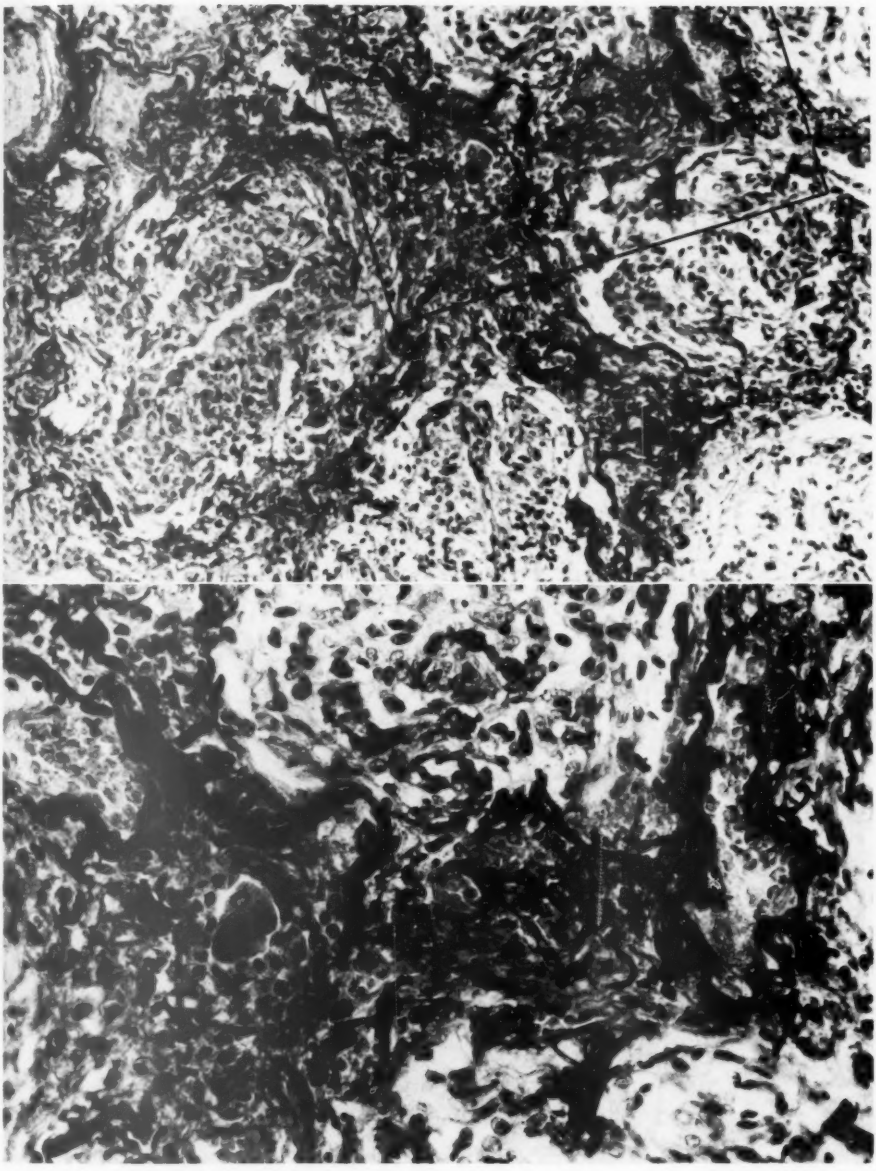

FIG. 7. Field B of Figure 5 at higher magnification. Small veins may be seen at the extremity of the nodule. Verhoeff elastic tissue stain. × 200.

FIG. 8. The field outlined in the oblong in Figure 7 at higher magnification to demonstrate small venules breaking through the elastic barrier to come into intimate relationship with the tumor cells. Verhoeff elastic tissue stain. × 400.

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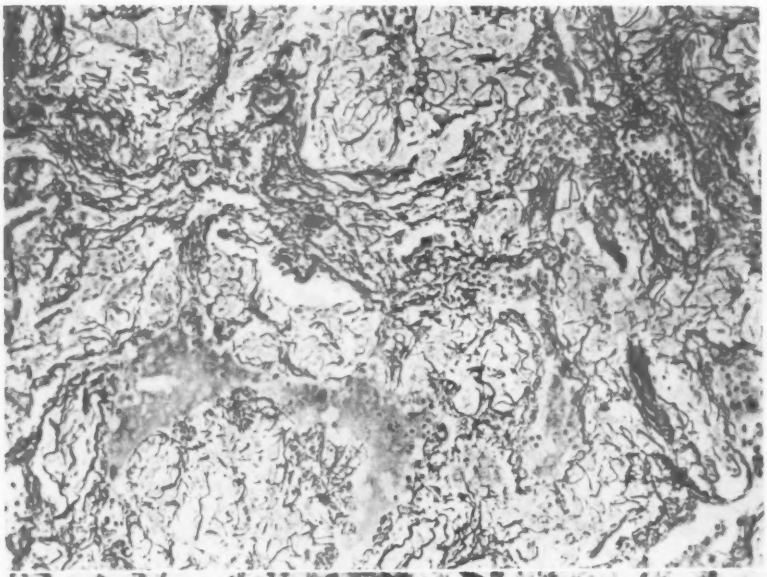

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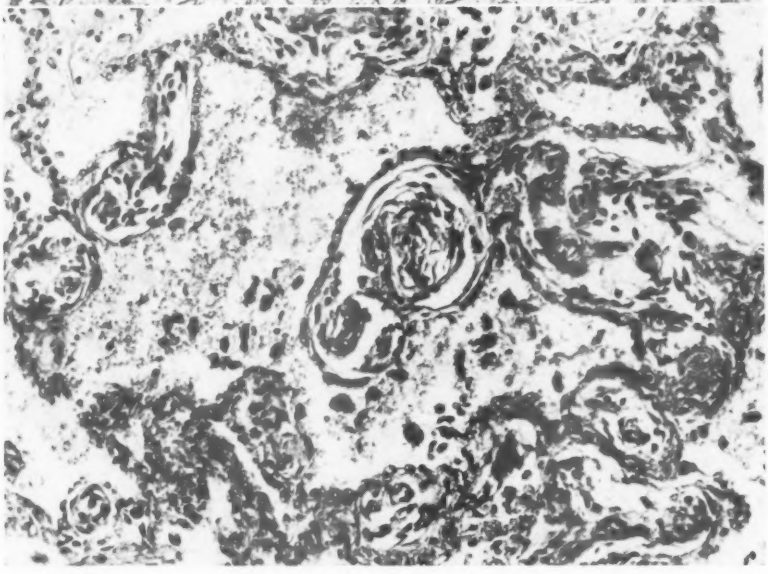

FIG. 9. Case 2. Reticulin outlines the *Zellballen*. Individual cells of the latter do not have the concentric arrangement about vessels characteristic of hemangiopericytoma. Gordon and Sweets stain. × 140.

FIG. 10. Case 15. A small lesion, the cells of which penetrate into the interalveolar septums. A barrier of capillaries is interposed between the groups of tumor cells and the lumens of the air spaces. × 175.

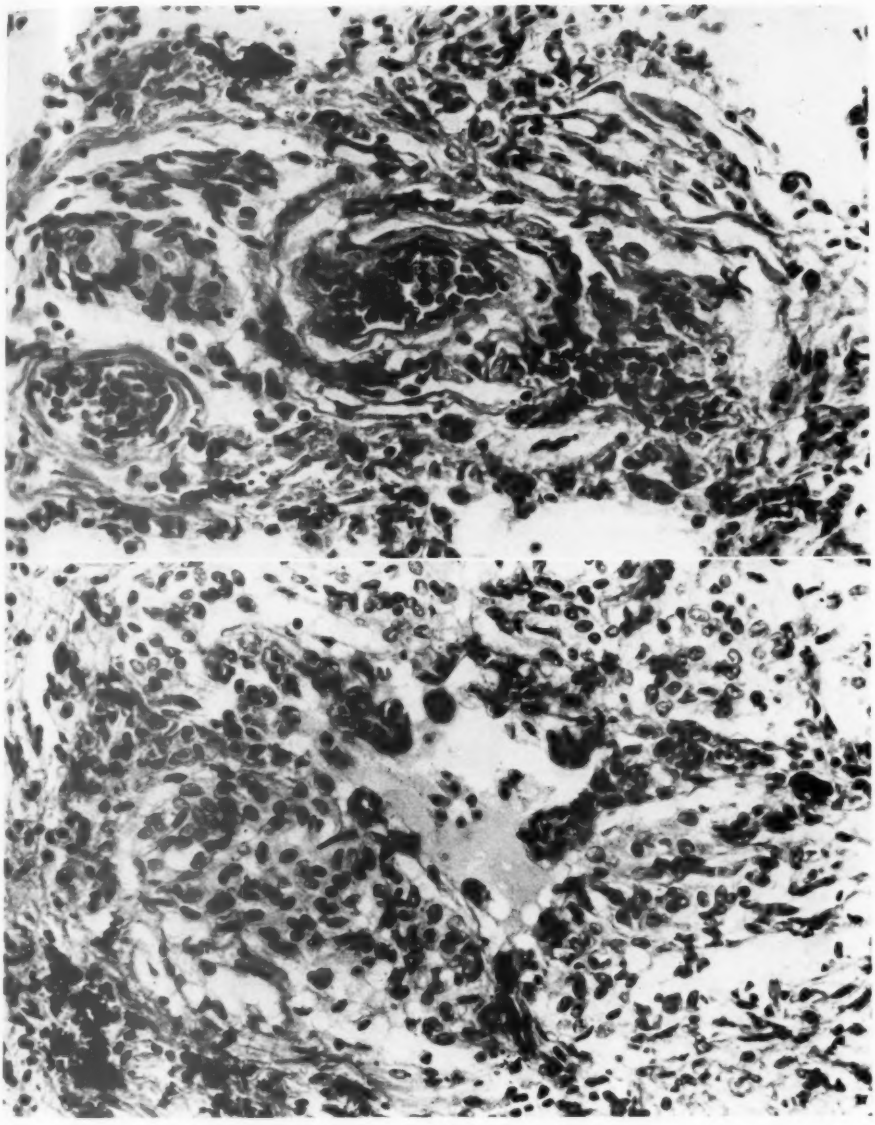

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FIG. 11. Case 7. A minute lesion lying within a pulmonary septum in the adventitia of a venule. This relationship may not be apparent peripherally in large tumors that invade the interalveolar septums, but must be established near the center of the lesion. × 400.

FIG. 12. Case 2. Narrowed residual air space containing an exudate with a few mononuclear cells. Its walls are infiltrated by tumor cells which are separated from its lumen by a thin layer of connective tissue containing capillaries. × 400.

FIG. 13. Case 2. Arterial supply of a small pulmonary chemodectoma. Camera lucida drawings on translucent paper were accurately superimposed and photographed by transillumination to provide the reconstruction. The tumor is shown in outline at the left. A tortuous pulmonary artery at the right gives a small arteriole (arrow) to the lesion. Compare with Figure 14.

FIG. 14. One of the fields drawn in preparing Figure 13. The arteriole enters the lesion and is distributed into capillaries. Its branchings correspond to those shown in the upper portion of Figure 13. The arrow marks the same arteriole denoted in Figure 13. Verhoeff elastic tissue stain. × 38.

FIG. 15. Case 2. Reconstruction of another lesion. The dotted lines indicate the outline of the tumor. The artery is shown as it enters the lesion. Capillaries arising from this vessel are distributed within the tumor.

FIG. 16. Case 18. Vinylite cast. A venule near the lower margin is branched in an unusual forklike fashion (arrow). Small anastomosing venules join the branches. × 2.5.

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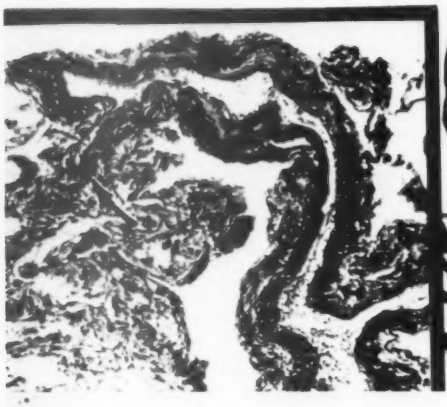

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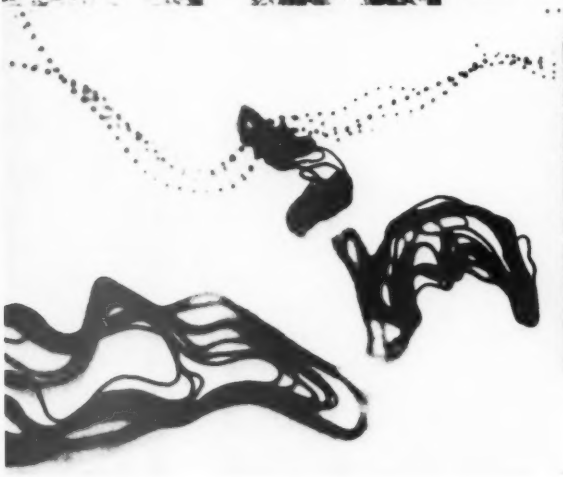

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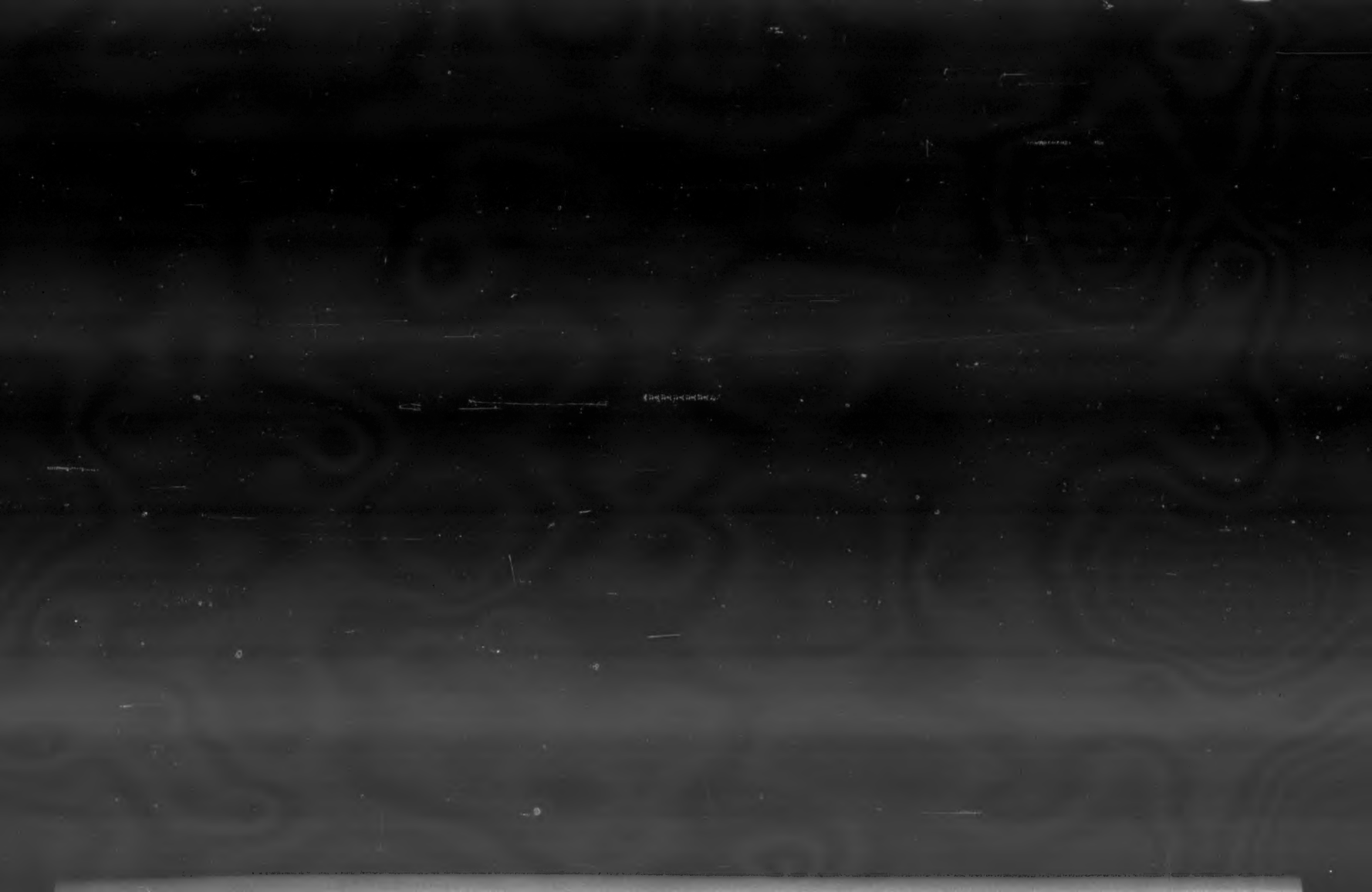
FIG. 17. Case 5. Pulmonary chemodectoma. The field contains a predominance of "usual" cells (LeCompte). Compare with carotid body tumor shown in Figure 20. Periodic acid-Schiff stain. × 350.

FIG. 18. Pulmonary chemodectoma. The field contains a predominance of spindle-shaped and fibrillar "angioma-like" cells (LeCompte). Compare with Figure 21. × 400.

FIG. 19. Pulmonary chemodectoma. The appearance of the component cells is intermediate between the "usual" and "angioma-like" patterns (LeCompte). Compare with Figure 22. × 400.



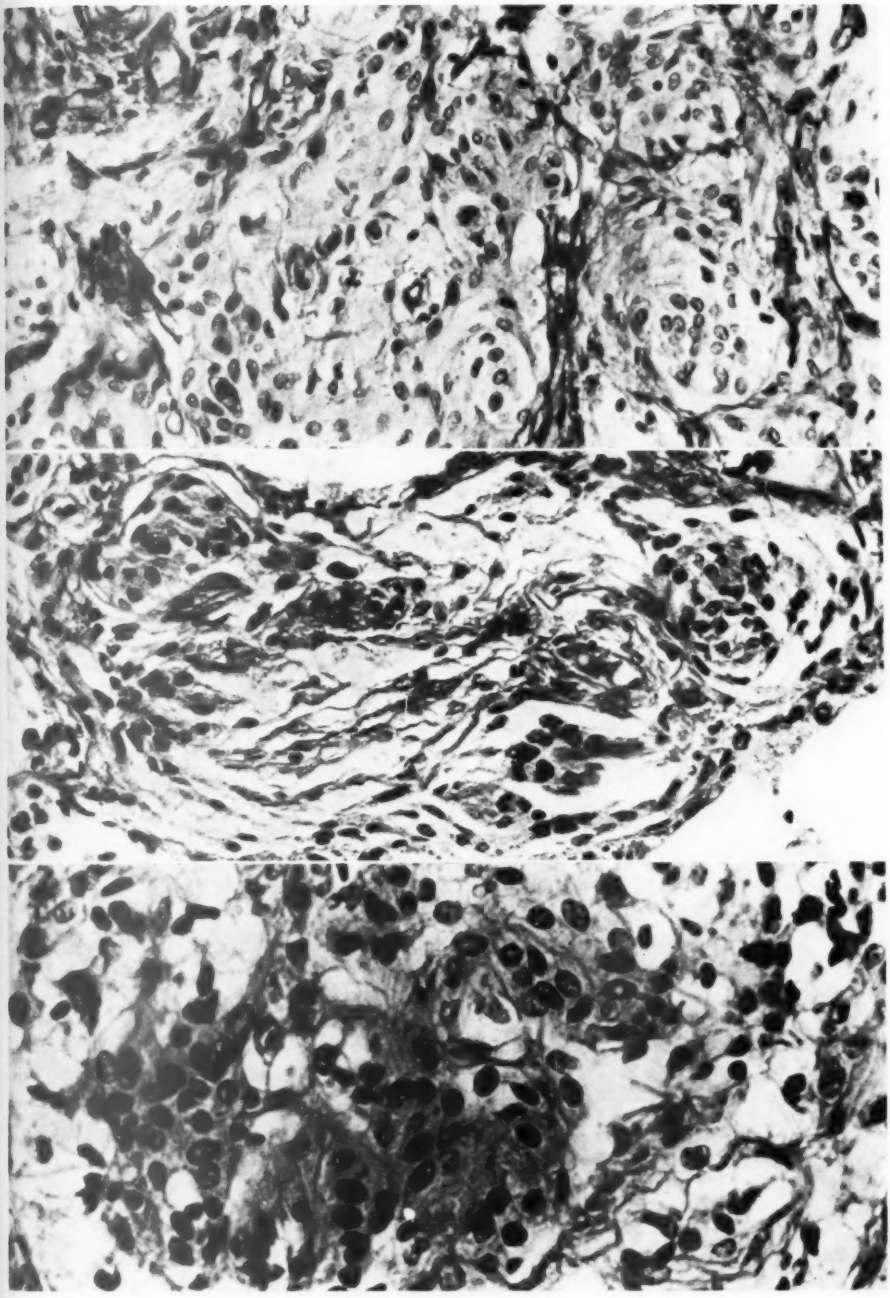


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FIG. 20. Tumor of the carotid body (Yale A 13458). At the right, "usual" cells predominate. At the left there is a predominance of "angioma-like" cells. × 175.

FIG. 21. Carotid body tumor metastatic in lung. Lesion is from the same patient shown in Figure 20. Predominance of "angioma-like" cells. × 175.

FIG. 22. Carotid body tumor metastatic in lung. Patient as in Figure 20. "Usual" as well as "angioma-like" cells. × 400.

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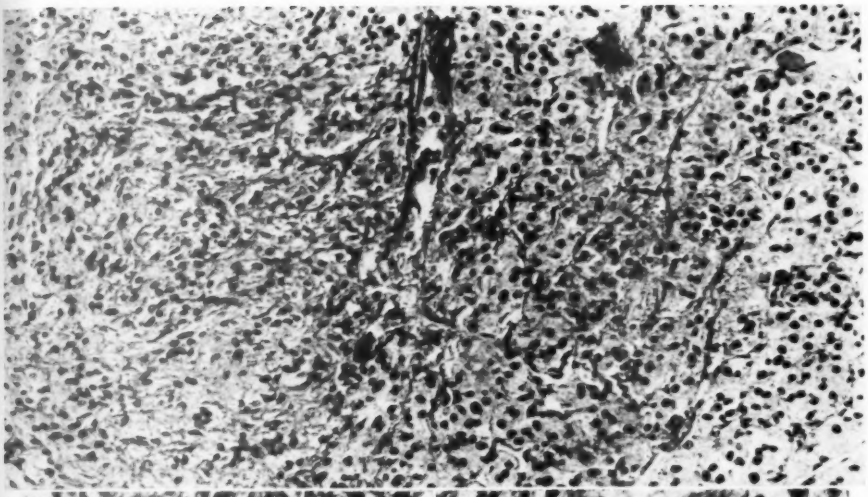

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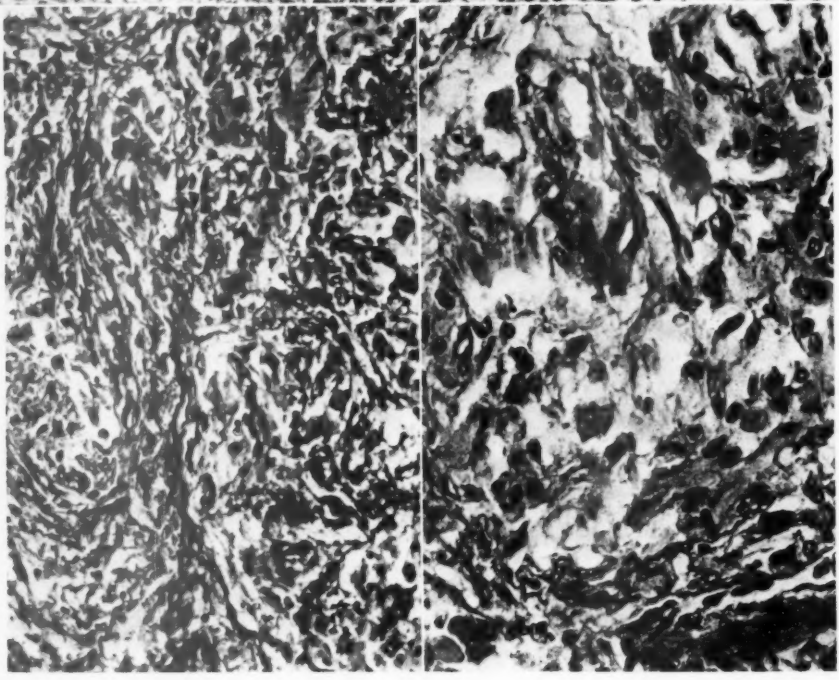

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FIG. 23. Case 19. Pulmonary chemodectoma. There is whorling of flattened cells about a minute central eosinophilic body. × 400.

FIG. 24. Case 2. Pulmonary chemodectoma. A mass of granular eosinophilic material appears at the center of a group of tumor cells. × 350.

FIG. 25. Case 19. Pulmonary chemodectoma. Fibrillar material that takes collagen stain is associated with flattened cells. × 400.

FIG. 26. Case 2. Pulmonary chemodectoma. Deposits of anthracotic pigment are limited to the vicinity of the veins and are never present within tumor cells. The reticulum stain accentuates the pattern of the *Zellballen* and outlines capillaries which form delicate vascular capsules for the tumor cells. Gordon and Sweets stain. × 180.

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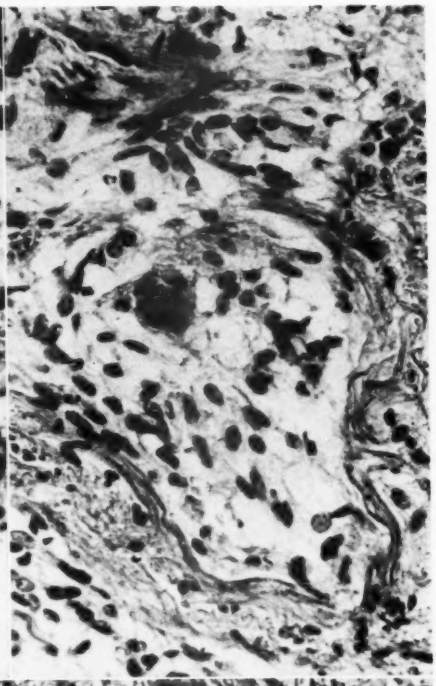

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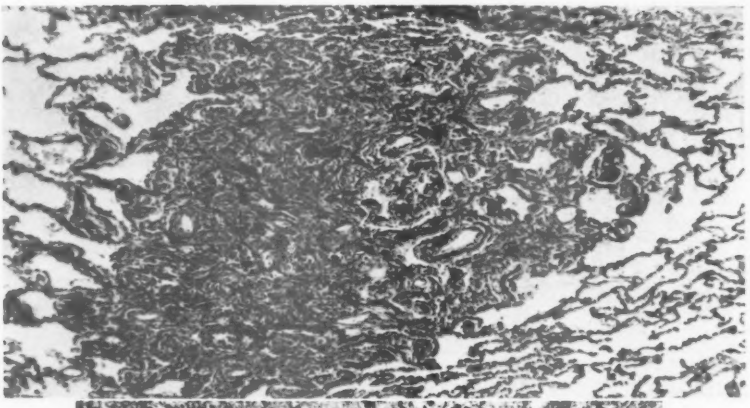

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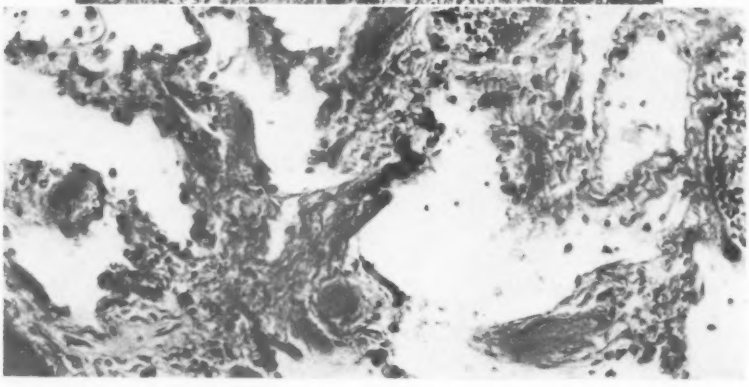

FIG. 27. Case 18. Pulmonary chemodectoma, partly hyalinized. The vein upon which the lesion is based and several of its branches are present in the section. Pigmented pleura is seen at the top. × 60.

FIG. 28. Case 18. A pulmonary chemodectoma with almost total hyalinization. Note the relationship to a vein. × 45.

FIG. 29. Case 12. Hyalinization of a tiny lesion. The relationship to the air spaces is similar to that of the lesion illustrated in Figure 10, and the interstitial position is clearly shown. × 175.

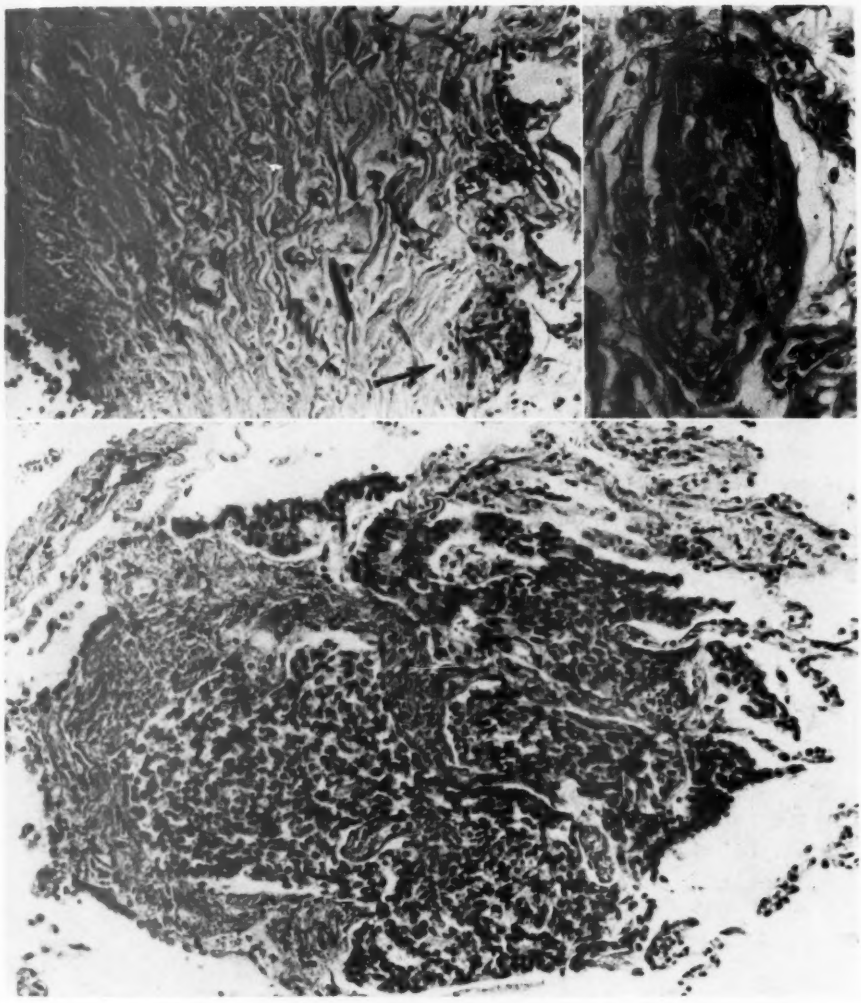

FIG. 30. A "normal" structure resembling pulmonary chemodectoma (Yale A 1237). A collection of cells (arrow) in the adventitia of a large vein. The entire wall of the latter is shown with a portion of the lumen in the left lower corner. Near the cell cluster are a nerve (right upper corner), a group of minute, presumably systemic arterioles, and some thinner-walled vessels thought to be venules. × 60.

FIG. 31. Close-up of structure shown in Figure 27 at another level of section. There is some resemblance to the component cells of the pulmonary chemodectoma. × 350.

FIG. 32. Atypical proliferation of epithelium (MGH 21377). This fits the description of a "tumourlet."[28] The cells partly line one air space (above) and fill the lumens of others. × 200.

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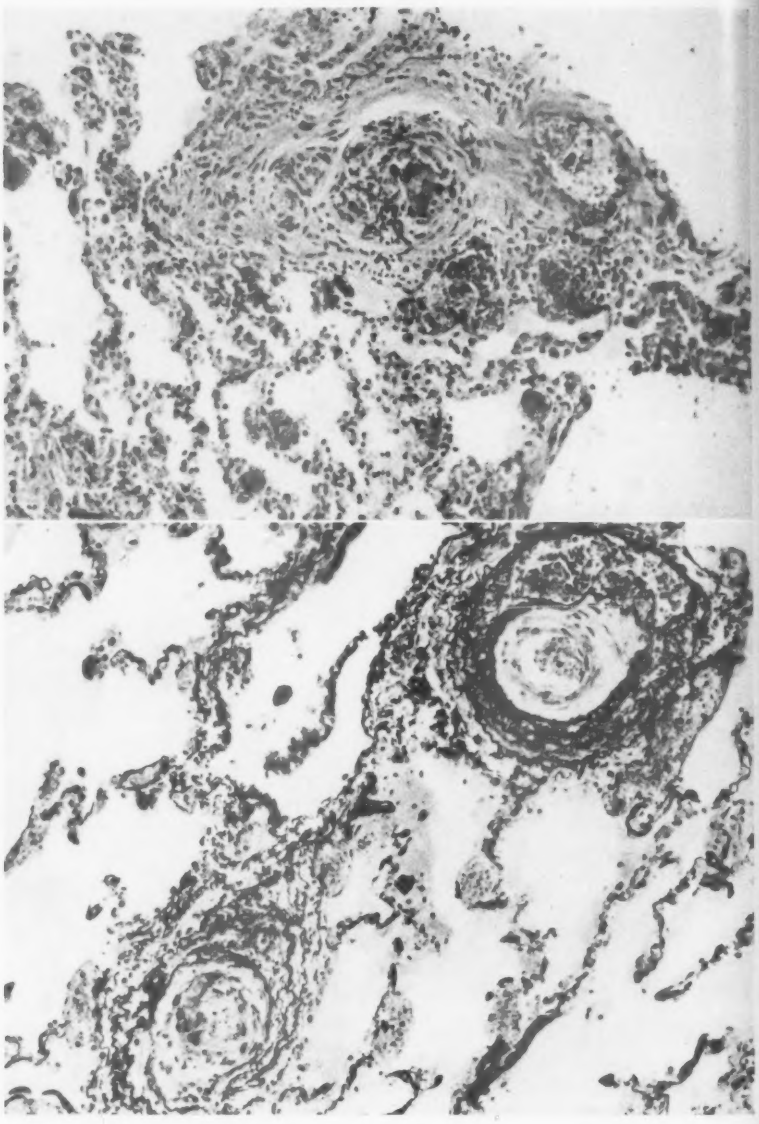

FIG. 33. Angiomatoid lesion from a case of pulmonary hypertension (MGH 13769). The lumen of the artery is filled with a mass of vasoformative tissue. One of the small, newly formed channels within this tissue contains a recent clot. × 140.

FIG. 34. Angiomatoid lesions from the case shown in Figure 33. The artery in the lower left corner presents a tuft of proliferating angiomatous tissue which is limited to the vessel lumen. Both the internal and external elastic laminas are intact. In the upper right corner an artery (probably the same vessel) shows intimal thickening. A small mass of vasoformative tissue occupies the media of the artery and is clearly delimited by elastic laminas. Verhoeff elastic tissue stain. × 140.

THE DISTRIBUTION OF EXPERIMENTAL AUTO-ALLERGIC LESIONS

Its Relation to the Distribution of Small Veins

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In recent years, several distinct and reproducible disease entities have been described in experimental animals immunized with autologous or homologous tissue constituents, usually injected with the Freund adjuvant (killed mycobacteria in mineral oil).[1] In each case, following a latent period of 10 days or longer, foci of inflammation and parenchymal destruction appear in the tissue corresponding to that used for immunization. Lesions have been induced in this manner in the central nervous system (encephalomyelitis), peripheral nervous system (polyneuritis), anterior segment of the eye with the use of lens antigen (phaco-anaphylactic endophthalmitis), posterior segment with uveal antigen (uveitis), testis, thyroid, and adrenal. These processes are strikingly similar in many of their experimental properties. On both immunologic and pathologic grounds, they appear to represent a single type of immunologic reaction, probably of the delayed or tuberculin type, to tissue auto-antigen.[1] They have, therefore, been provisionally designated as "experimental auto-allergies."

The primary determinant of the pattern of lesions in each auto-allergy is the distribution in the tissues of the antigen to which the animal has been sensitized. Thus in the rabbit or guinea pig sensitized to central nervous system myelin, lesions occur throughout the white matter of the brain, cerebellum, spinal cord, optic nerves, and even in that portion of the retina where there are myelinated nerve fibers. Lesions are minimal in the gray matter, where there is little myelin, and are lacking elsewhere. Similarly, in auto-allergic disease of the eye, only that part of the eye is affected which relates to the antigen utilized, the lens in one instance, the uvea in the other. Nevertheless, in several of these experimental conditions, there are obvious sites of predilection, where lesions occur in greater number and appear to be more intense than in other

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* National Neurological Research Foundation Scientist.

parts of the antigen-bearing tissue. Again, as an example, allergic neuritis in the rabbit primarily affects the dorsal spinal roots and sensory ganglia, though the auto-antigen is present not only in these sites but throughout the peripheral nerve as well.

It has been shown [2] that in the peripheral nervous system the regions in which auto-allergic lesions occur preferentially are, in fact, areas in which colloidal anionic dyes, such as trypan blue, labeled anionic proteins (i.e., serum albumin and diphtheria toxin) and colloidal silver readily pass from the blood stream into the nerve parenchyma. These are also areas where the vascular network includes numerous small or moderate-sized vessels of venous type. From this circumstantial evidence we drew the tentative conclusion that the occurrence of auto-allergic lesions may depend on the presence of vessels of proper type, and that the anatomic or functional properties of veins, in particular, permit the expression of this type of hypersensitive reaction.

In the present report, evidence is presented to show that in other organs, as well, the eye, the testis, and the adrenal in particular, the sites of predilection for the occurrence of auto-allergic lesions are zones in which preferential passage of anionic colloids occurs and in which there are conspicuous vessels of venous type.

Methods

Histologic materials from animals with each type of disease process mentioned (with the exception of auto-allergic uveitis) were prepared in our own laboratory or obtained from laboratories where they were under active investigation.* The technique of producing disease, in almost all instances, consisted of one or several intradermal or foot-pad injections of homologous tissue homogenized in mineral oil containing heat-killed tubercle bacilli, with or without the addition of an emulsifying agent. Where possible, tissues were examined from more than one species of experimental animal. Paraffin sections stained with hematoxylin and eosin and, in some instances, with other special stains (e.g., for myelin) were examined microscopically. The detailed histologic features have been presented elsewhere.[1] Here only data having to do with lesion distribution are considered.

To demonstrate sites of passage of colloidal substances from the blood stream, normal rabbits and guinea pigs received intravenous or subcutaneous injections of relatively large doses of dyes of differing molecular size and charge or of I^{131}-labeled proteins. Others received 0.15 per cent silver nitrate in their drinking water for periods of 12 to 15 months. Silver was presumably transported in the blood stream in colloidal form.[3] The methods for demonstrating dye, radioactivity, or silver in tissue sections have been described in detail.[3] Only data obtained with Niagara sky blue 6B (a colloidal anionic dye) and with I^{131}-labeled human serum albumin (Abbott) are included in the present report, except where indicated otherwise.

Vessels were examined in frozen sections of various tissues stained by the Pickworth (benzidine) technique.[4] Adequately stained preparations from at least 3 animals were examined in each case.

* We are grateful to Dr. E. Witebsky and his collaborators for the loan of sections of dog, rabbit, and guinea pig thyroids affected by allergic thyroiditis, to Dr. J. Colover for sections demonstrating allergic adrenalitis in the guinea pig, and to Dr. L. Zimmerman for slides showing phaco-anaphylactic endophthalmitis in the rabbit.

TABLE I

COMPARISON OF INTENSITY OF LESIONS AND PASSAGE OF COLLOIDS IN VARIOUS REGIONS IN THE NERVOUS SYSTEM *

Tissue element	Auto-allergic lesions †		Staining by dye		Passage of protein		Deposition of silver	
	Rabbit	Guinea pig	Rabbit	Guinea pig	Rabbit	Guinea pig	Rabbit	Guinea pig
Meninges	+++	+++	+++	+++	+++	+++	++	++
Gray matter	o—+	o—+	o—+	o	+	+	o	o
White matter	o—++	o—++	o	o	o	o	o	o
Posterior roots and spinal ganglia	+++	+	++	+—++	+++	++	+	+
Epineurium and perineurium	o	+	+++	+++	+++	+++	++	++
Sciatic nerve parenchyma	o—+	+++	o	++	o	++	o	o

* Data abstracted from: Waksman, B. H. Experimental study of diphtheritic polyneuritis in the rabbit and guinea pig. III. The blood-nerve barrier in the rabbit.[8]

† Experimental allergic encephalomyelitis and neuritis.[6]

TABLE II

COMPARISON OF INTENSITY OF LESIONS AND STAINING BY NIAGARA SKY BLUE 6B IN VARIOUS ELEMENTS OF THE EYE

	Tissues remaining unstained					Tissues stained by dye			
	Auto-allergic lesions		Degree of staining			Auto-allergic lesions		Degree of staining	
	Rabbit *	Guinea pig †	Rabbit	Guinea pig		Rabbit	Guinea pig	Rabbit	Guinea pig
Cornea	+ (near limbus)	o	o	o	Iris	+++	+	+—++	±
Lens	o	o	o	o	Ciliary body	+++	++	++	+++
Vitreous body	o	o	o	o	Choroid	o	+++	+++	+++
Retina	o	o	o	o	Sclera	o	o	±	+
Optic nerve head	+	+	o	—	Scleral limbus	++	o	++	+++

* Phaco-anaphylactic endophthalmitis.

† Experimental allergic uveitis.

Results

Nervous System[2,5]

The passage of colloidal anionic dyes (Niagara sky blue 6B, trypan red, trypan blue), or radio-labeled acidic proteins (human serum albumin, diphtheria toxin), and of colloidal silver into various parenchymal and connective tissue elements of the nervous system is shown in Table I and compared to the intensity of the lesions of auto-allergic encephalomyelitis and neuritis in these areas. The designations o—+ and o—++ are meant to indicate that the gray and white matter of the spinal cord, in some animals, remained free of lesions even in the presence of severe meningitis, while in others, lesions were present but only in isolated perivenous foci. Similarly, isolated foci occurred in the sciatic nerve of the rabbit, but, more frequently, lesions were entirely absent. It was clear that the posterior roots and sensory ganglia in the rabbit, the peripheral nerve in the guinea pig, and the meninges of both species, preferred sites for the occurrence of lesions, were also sites for preferential passage of the large molecules investigated.

In sections stained by the Pickworth technique,[4] in both species, the spinal cord white matter, in addition to the capillary network, contained a limited number of radially oriented veins. There were many medium-sized and large veins in the meninges. Few veins (15 to 20 μ) were seen in the spinal ganglia of the guinea pig. There were many more in the rabbit ganglia; here, also, there were ampulla-like vascular dilatations (up to 30 μ) in the central gray matter. In the rabbit's sciatic nerve, the endoneural vascular plexus was made up almost exclusively of capillaries, while in the guinea pig a considerable proportion of the endoneural vessels were larger than capillaries (11 to 25 μ); these were regarded as being venous in character since they did not have a muscular coat. The vessels were comparable in number in the two species, being most numerous in cellular areas of the spinal cord and sensory ganglia. These observations were confirmed by examination of the vascular network in these tissues by other methods (injection with India ink-gelatin, impregnation of vascular endothelium with lead, counting of veins in hematoxylin and eosin-stained cross sections of nerve).[2]

Eye

In Table II the relative staining of different parts of the eye with the colloidal anionic dye, Niagara sky blue 6B, is compared with the relative intensity of lesions in these areas. Figures 1 and 2 show the highly selective nature of the staining observed in rabbit and guinea pig eyes. Again it is clear that the inflammatory response occurred prefer-

entially in those tissues which were readily stained by the dye. Lens antigen primarily elicited a reaction in the iris, ciliary body and corneal limbus; uveal antigen produced a reaction in the choroid.[1]

Acid dyes, even of large molecular size, were found to pass more readily into the aqueous humor than into the cerebrospinal fluid (Table III). This was in essential agreement with the observations of others.[7]

TABLE III

PASSAGE OF DYES OF DIFFERENT MOLECULAR SIZE AND CHARGE INTO CEREBROSPINAL FLUID AND AQUEOUS HUMOR

Dye	Time after injection (hr.)	Intensity of staining of		
		Rabbit spinal fluid	Rabbit aqueous humor	Guinea pig aqueous humor
Nondiffusible anionic				
Trypan red	4	o	+	+++
	23	+	++	−
Niagara sky blue 6B	2	+	++	+++
Diffusible anionic				
Patent blue V	1	+	+++	+++
	4	++	+++	+++
Neutral				
Neutral red	2	+++	+	−
Cationic				
Brilliant cresyl blue	2	+++	o	−
Methylene blue	2	o	o	o
	5	o	o	o
Nile blue A	1	o	o	o
	4	o	o	−
Crystal violet	5	o	++	−
New fuchsin	5	o	o	−

Both media behaved as connective tissue spaces in that their staining with these dyes paralleled the behavior of the meninges and ciliary body respectively. Basic dyes apparently become fixed in the tissues and do not, in general, make their way into the subarachnoid space and the anterior chamber.

In the guinea pig eye (Figs. 3 and 4) the choroid was found to consist largely of a network of venous sinusoids 10 to 25 μ in diameter. The ciliary body vessels measured 8 to 20 μ; a few were smaller. The iris had radially oriented veins on its anterior surface; these measured 10 to 25 μ in diameter, most being about 12 μ. While the sclera contained only capillaries, several veins (8 to 15 μ) formed a plexus at the corneoscleral limbus; infrequently one of these extended some distance over the anterior surface of the cornea. The rabbit eye appeared similar except that the choroid sinusoids measured 20 to 60 μ in diameter, the ciliary

body veins about 15 μ, and the iris vessels, located about midway between anterior and posterior surfaces, 20 to 30 μ. Few large limbal vessels were seen.

Testis

Experimental allergic orchitis in the guinea pig preferentially affects the epididymis and the rete testis[1] (Figs. 5 and 9). With both Niagara sky blue 6B and radio-labeled human serum albumin there was preferential staining in these regions of the guinea pig testis (Table IV; Figs. 6 and 7). As indicated, there was considerable variation in the occurrence of lesions between the seminiferous tubules among the animals; a similar variation was found in the degree of passage of dye and labeled protein in regions remote from the rete. In guinea pigs with argyria, silver was deposited more or less uniformly in intertubular connective tissue throughout the testis.

TABLE IV

COMPARISON OF LESION INTENSITY WITH PASSAGE OF NIAGARA SKY BLUE 6B AND I^{131}-LABELED HUMAN SERUM ALBUMIN IN VARIOUS REGIONS OF GUINEA PIG TESTIS

Tissue element	Auto-allergic lesions	Dye	Protein
Epididymis	+++	++	+
Seminiferous tubules	o	o	o
Connective tissue between tubules	+−++	o−+	±−+
Rete testis	+++	++	+++
Capsule and fat	−	+++	+++

In the guinea pig testis, large veins (more than 100 μ in diameter) are present in the capsule and the rete. The latter branch to give vessels measuring about 20 μ, one for every 10 to 20 seminiferous tubules; these in turn subdivide to give venules (9 to 15 μ, not more than one per 3 to 4 tubules) and capillaries. In parts of the epididymis, small veins and venules are much more numerous than among the seminiferous tubules, and vessels measuring 15 to 20 μ are quite numerous (one per 3 to 4 tubules). Figure 10 illustrates the difference between the two regions of the testis.

Thyroid

The lesions of experimental allergic thyroiditis may be focal or quite diffuse (Fig. 11).[1] Accordingly, no pattern of relative staining could be ascertained in the rabbit or guinea pig thyroid. With Niagara sky blue 6B, the capsule stained somewhat more intensely than the parenchyma of the gland.

In the guinea pig and rabbit thyroids, all follicles are richly invested with capillaries. The venous network is seen throughout the gland, ves-

sels measuring 200 μ branching repeatedly to form vessels measuring 20 to 40 μ in the connective tissue septums and still smaller veins in most, but not all, of the lobules. Large vessels were not seen in the capsule (Fig. 12).

Adrenal

The lesions of auto-allergic adrenalitis were localized in the inner zone of the adrenal cortex; only a few lesions were found in the medulla as well (Fig. 13).[1,8] This distribution is exactly comparable to the clear-cut and reproducible staining pattern of the adrenal, indicated in Table V and Figures 14 and 15. It was further confirmed by radioautographs of the adrenal in animals injected with labeled serum albumin (Fig. 16). There was a gradual increase in staining toward the inner portion of the zona fasciculata, and some lesions were found in this area. Lesions were numerous and staining intense in the zona reticularis. The zone of uptake of radioactivity at the corticomedullary junction was narrower than the zone of staining after injection of Niagara sky blue 6B.

The rabbit and guinea pig adrenals presented essentially identical vascular patterns. The medullary sinusoids measured up to 50 μ in diameter. In the zona glomerulosa of the cortex, there was a network of vessels measuring between 8 and 12 μ in diameter with occasional prolongations inward toward the medulla. The zona fasciculata contained only radially oriented capillaries; ¾ of these measured 3 to 6 μ and the

TABLE V

COMPARISON OF INTENSITY OF LESIONS WITH PASSAGE OF NIAGARA SKY BLUE 6B AND I^{131}-LABELED HUMAN SERUM ALBUMIN IN VARIOUS REGIONS OF GUINEA PIG ADRENAL

Tissue element	Auto-allergic lesions	Dye	Protein
Medulla	o—+	+	+
Cortex			
Glomerulosa	o	+	o
Fasciculata	o—+	o—+	o
Reticularis	+++	+++	++
Capsule	o	+++	+++

remainder measured 6 to 10 μ. In the zona reticularis, these vessels enlarged to 10 to 12 μ and rapidly increased in size to form tortuous veins, about 25 μ in diameter, at the corticomedullary junction (Fig. 17).

DISCUSSION

An essential element in the pathogenesis of disease is that complex of host factors, not necessarily part of the etiologic mechanism, which determines the distribution of lesions. For allergic processes, such factors

have not till recently received much attention. The pathophysiologic effects of anaphylaxis, for example, are determined by the release of histamine and other pharmacologically active substances and their action on smooth muscle and vascular permeability. The localization of lesions in this instance, therefore, depends to a significant extent on the distribution of cells from which such substances are released and upon the distribution of smooth muscle.[9] An additional factor is the localization of antibody, which becomes reversibly fixed in the tissues.[10,11] Benacerraf, Biozzi and Halpern [12] have recently shown that nonspecific local inflammation facilitates local antibody fixation and may thereby determine the later distribution of the specific anaphylactic lesion. Similarly, in the case of certain lesions of the Arthus type, produced by circulating antigen-antibody complexes, the distribution is determined in part by the ability of these complexes to cause histamine release in the circulation and the effect of the histamine in altering the phagocytic properties of the vascular endothelium in particular local sites.[13,14] The present paper deals with the problem of localization in a third category of allergic disorders, the so-called "experimental auto-allergies," which appear to be disseminated reactions of the delayed hypersensitivity type to antigen normally present in the tissues.[1] In these, the available evidence suggests that the reaction consists mainly of an interaction of circulating mononuclear cells with the extravascular antigen.[15] The cells leave the blood stream and react further in an as yet unknown manner.

Our experimental findings are essentially consistent in showing that the areas in which cellular inflammation is particularly prominent in the various auto-allergies are areas in which there is unusually free passage of the anionic dye, Niagara sky blue 6B, and of labeled anionic proteins, and in which venules and veins are numerous.* These regions include the meninges, the dorsal nerve roots and ganglia (rabbit) or peripheral nerve parenchyma (guinea pig), the choroid, ciliary body and iris, the epididymis and rete testis, and the zona reticularis of the adrenal cortex. Our data derived from observations of chronic silver deposition are fragmentary, but sufficient information is available from other sources to show that silver deposits in argyria tend to follow a similar distribution.[16–19] More specifically, in the central nervous system, the meninges and choroid plexus and certain other limited areas such as the pituitary show deposits.[18] In the peripheral nervous system, silver is seen only in the epi- and perineurium.[2,16] In the eye, aside from the cornea and con-

* The present study has not concerned itself with the passage of dye or other colloids from the blood stream in tissues where auto-allergies have not been shown to occur. In muscle and skin, for example, where the vessels may be of the correct type, it is nevertheless impossible to induce lesions in the absence of a suitable auto-antigen.

junctiva, deposits are found in the ciliary processes and the choroid; the lens, vitreous, retina and optic nerve are spared.[19] Silver is said to be deposited in the capsular and vascular connective tissue of the testis, in the follicular basement membranes and connective tissue of the thyroid, and in the capsule and connective tissue septums of the adrenal.[17]

The estimated intensities of staining in various tissues following parenteral administration of dye have, of course, no absolute significance. The relative intensities within a single organ, on the other hand, should provide a valid measure of differences of *in vivo* permeability of vessels between different regions of the given organ. These differences were quite consistent from animal to animal in the present study. Similarly, results obtained with the Pickworth staining method must be interpreted with caution since the correctness of estimates of vessel size depends on the adequacy of filling of the vascular tree in any area investigated. Our data with regard to the peripheral nervous system [2] were confirmed with the use of other methods (India ink-gelatin injection, lead impregnation of endothelial "cement"). In each of the other organs investigated, it was possible to examine sections in which the entire vascular tree was well-filled and easily visualized; the findings for each tissue were duplicated in sections from several animals. The results were clear-cut and agreed with published descriptions in each case.[20]

The question now arises as to the significance of these observations. In a paper on the blood-nerve barrier,[2] the experimental evidence supporting the view that veins are distinguished from arterioles and capillaries by anatomic differences was reviewed. These determine preferential passage, not only of large molecular dyes and other colloids [21–23] but also of inflammatory cells.[24–26] The nature of their pertinent structural features is unknown. It is customary to assume that there may exist important differences in the number or size of endothelial pores.[21–24,27,28] However, a satisfactory morphologic demonstration of these has not hitherto been available.[29] Cohnheim was led to the conclusion [25] that in inflammation some direct alteration of the vessel wall must occur to permit the passage of leukocytes.[30] Recently it has been shown [31] that under the influence of agents such as histamine, holes are produced in the endothelium of venules but not in arteries or capillaries. The data obtained in the present study constitute, at best, evidence of an indirect type, indicating the importance of veins or venules in permitting the expression of the auto-allergic mechanism. They do not permit one to identify direct alteration of the vascular endothelium, which may also play a role in the formation of the lesion.

In each form of auto-allergy investigated sufficiently from the histologic point of view, lesions appear to begin with perivenous inflamma-

tion [1]; this is a property of delayed hypersensitive reactions in general.[15] This well recognized histologic feature and the rather clear parallel between the presence of veins, the passage of colloids and the occurrence of diffuse or unusually intense lesions would appear to justify the inference that in areas containing antigen, the sites of predilection for auto-allergic lesions will in general be regions containing more or less numerous venules and veins. Where few veins are found, no lesions will occur, or, at best, these will be isolated perivenous foci. It should be stressed again, however, that the location of lesions in the auto-allergic states is principally determined by the location of antigen.[1] What we are concerned with in the present report is merely the identification of secondary localizing factors.

The data fail to provide any explanation for two well established features of the distribution of auto-allergic lesions. One is their focal character in areas where only occasional veins are present. This may depend on preferential localization at a site where lesions are already present—the blood-tissue barrier is strikingly reduced where parenchymal invasion by inflammatory cells has actually occurred.[32] Secondly, there is a marked increase in both the number and intensity of lesions in an area where a change in the blood-tissue barrier is experimentally induced, as shown both in the central nervous system [33,34] and the eye.[35] Here one cannot choose between the possibility that some capillaries have temporarily assumed, perhaps with vasodilatation, the functional properties of venules or the possibility that more veins have been made accessible. The latter is more probably correct, since it is quite unjustified to assume that the blood-brain and blood-eye barriers merely represent the absence of veins. On the contrary, there are veins in these organs, and the barrier, as attested by a variety of experimental evidence,[18,36–38] must depend on additional anatomic features.

Histologic and other studies of the auto-allergic states have indicated that this group of experimental disorders shares most or all of the histologic and immunologic features of delayed hypersensitive reactions in general. Data will be presented in later publications to show that the relationship described in the present study between veins or venules, the passage of anionic colloids from the blood stream, and the occurrence of lesions is in fact a characteristic feature of many other types of delayed lesions. Among these are the tuberculin reaction, the reaction of contact allergy, and the rejection of skin homografts. A simple example, underlining the general significance of this relationship, is the well known failure of the rat to manifest a tuberculin reaction in the skin of the flank. This animal does, in fact, develop tuberculin sensitivity, and tuberculin reactions may be elicited readily in the subcutaneous muscle

and fat and in such special skin areas as the ear. It has been found that a venular network is almost entirely lacking in the dermis of the rat's flank skin, and this area remains unstained after intravenous injection of trypan blue.

Summary

Auto-allergic lesions affecting the central nervous system, peripheral nervous system, lens, uvea, thyroid, testis, and adrenal occur more frequently and more intensely in certain sites of predilection. These are the meninges and subpial region of the spinal cord, the posterior spinal root and sensory spinal ganglion in the rabbit, or the peripheral nerve in the guinea pig, the choroid and ciliary body of the eye, the rete testis and epididymis, and the zona reticularis of the adrenal cortex. These are found to be sites of preferential passage of such dyes as trypan blue and of other anionic colloids (labeled human serum albumin). They are also characterized by the presence of conspicuous and, in some instances, numerous small and medium-sized veins.

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Indebtedness to Miss Christine Wennersten for her assistance in the technical phases of this project is gratefully acknowledged.

[*Illustrations follow*]

Legends for Figures

Fig. 1. Staining of the corneal limbus, base of the iris, ciliary body, and choroid in a normal guinea pig eye 40 minutes after the intravenous injection of Niagara sky blue 6B. Carnoy's fixative, paraffin section (32 μ). × 36.

Fig. 2. The optic nerve head in the eye of a rabbit 2 hours after the intravenous injection of Niagara sky blue 6B, showing intense staining of the choroid. Fragmentation of the retina is due to artifact. Frozen section (200 μ). × 80.

Fig. 3. Normal guinea pig eye, showing large veins of the choroid. Retina is to the right. Hematoxylin and eosin stain. × 125.

Fig. 4. Vessels in the normal guinea pig eye. The venous plexus of the ciliary body and veins of the iris and at the limbus of the cornea are shown. Frozen section (250 μ), Pickworth stain. × 40.

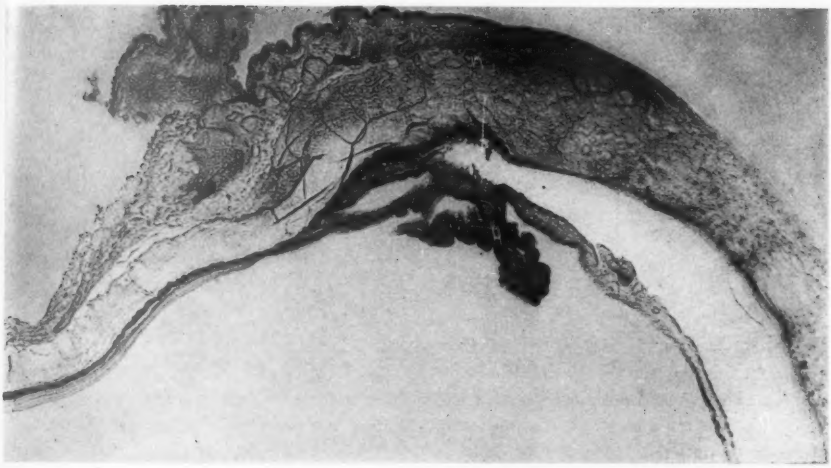

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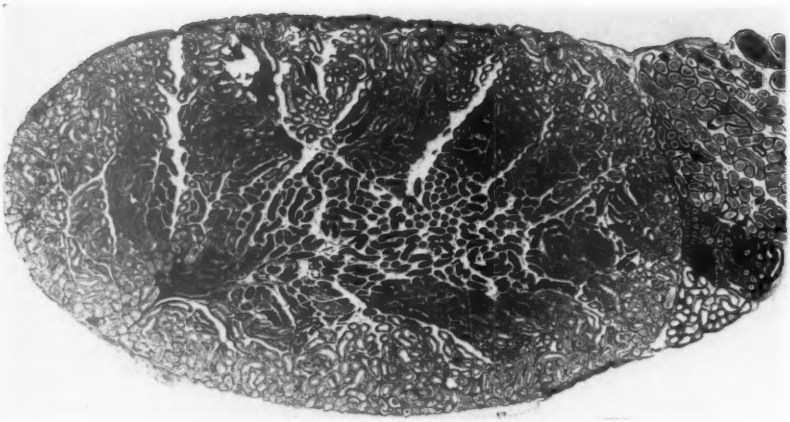
FIG. 5. Distribution of the lesion in acute auto-allergic orchitis in a guinea pig testis 12 days after inoculation (intradermal) with homologous testis plus adjuvant. Massive inflammatory lesions are present in the rete testis and in one area of the epididymis. Hematoxylin and eosin stain. × 6.5.

FIG. 6. Radioautograph of a guinea pig testis 18 hours after the intravenous injection of I^{131}-labeled human serum albumin. The label is present primarily in the capsule and the rete testis; to a lesser extent it appears in the central portion of the testis along larger branches of the rete veins and in the epididymis. × 6.5.

FIG. 7. Staining of connective tissue in the rete area of the guinea pig testis 20 minutes after the intravenous injection of Niagara sky blue 6B. Seminiferous tubules to each side are unstained. Frozen section (200 μ). × 80.

FIG. 8. Silver deposits in the intertubular connective tissue of the guinea pig testis after ingestion of silver nitrate (0.15 per cent) in drinking water for more than one year. Paraffin section, dark field. × 125.



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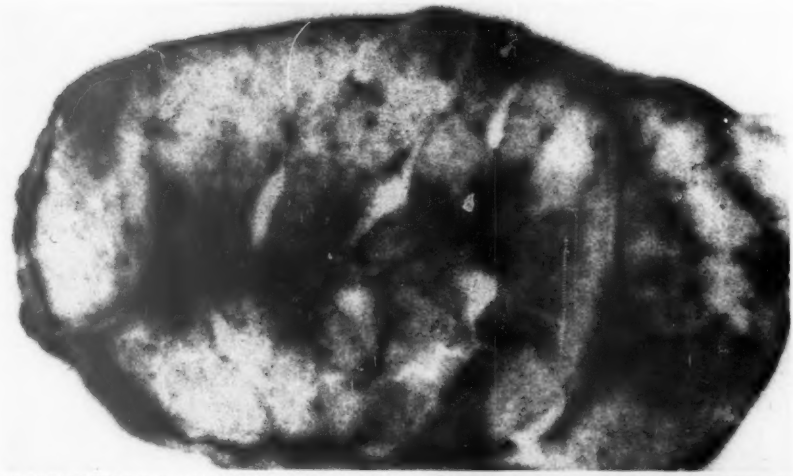

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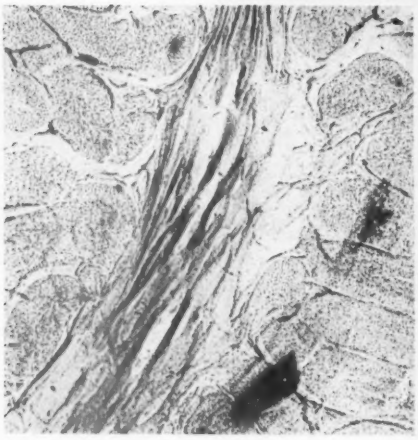

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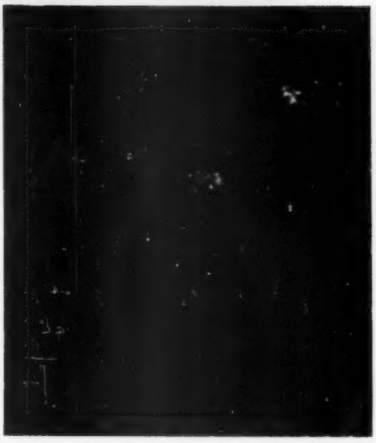

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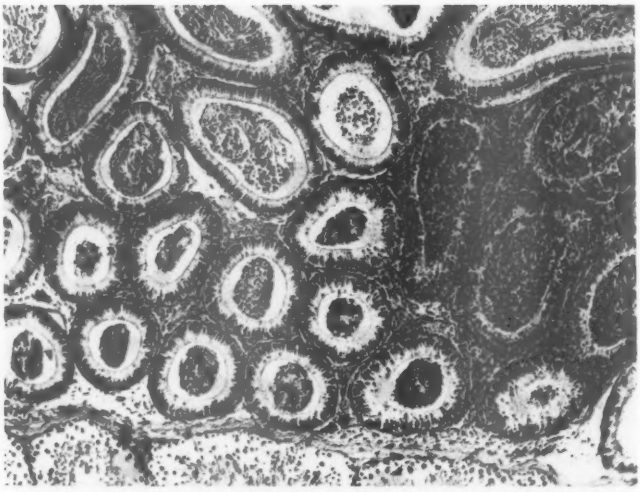

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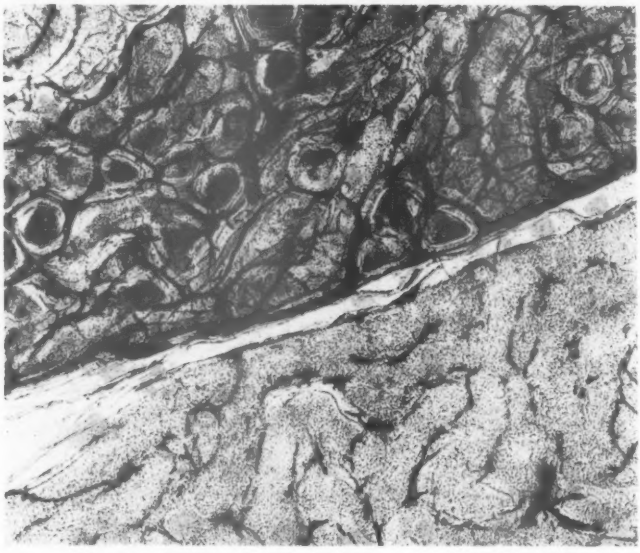
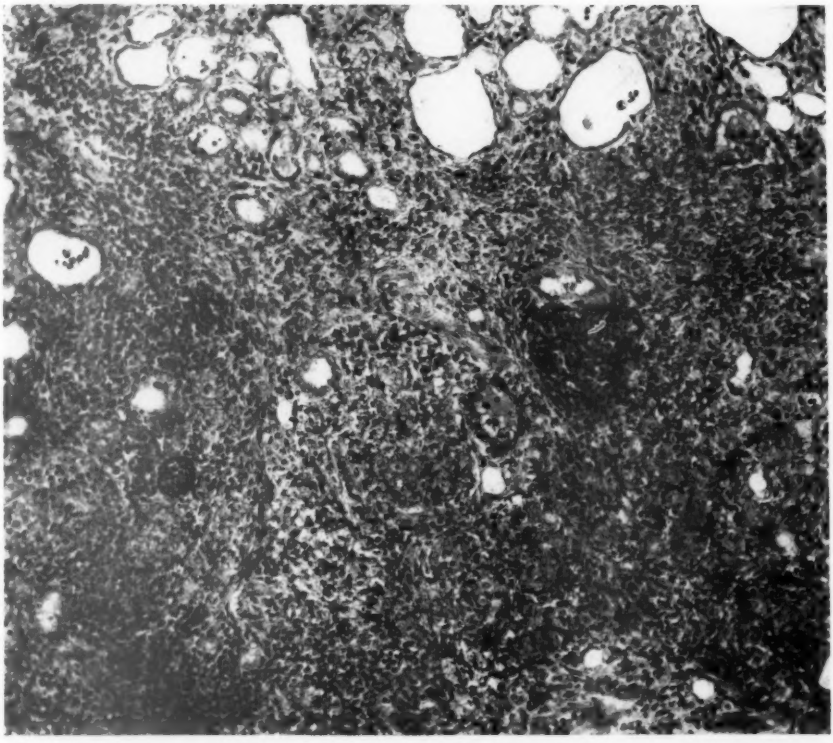


FIG. 9. Higher power view of the testis shown in Figure 5. No inflammation is present among the seminiferous tubules in the body of the testis, below. There is massive intertubular inflammation in the epididymis, above, with invasion of many tubules by inflammatory cells. Hematoxylin and eosin stain. × 80.

FIG. 10. Vessels in the normal guinea pig testis. Isolated veins are seen among the seminiferous tubules, below. Numerous veins are shown in the epididymis, above. Frozen section (250 μ), Pickworth stain. × 36.



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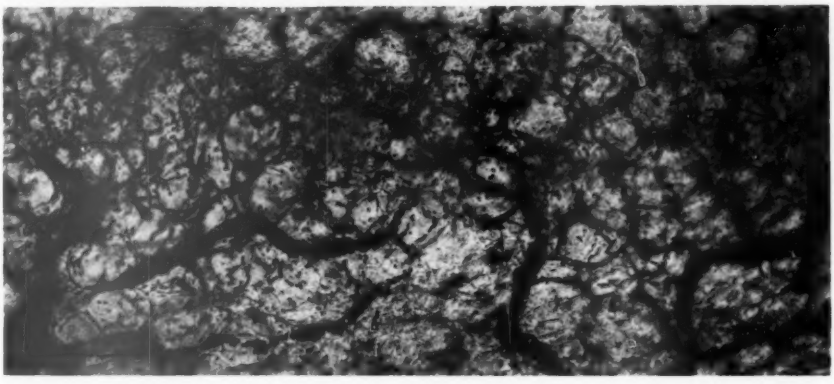

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FIG. 11. Auto-allergic thyroiditis in the rabbit. The diffuse character of the intense, long-standing lesion is shown. Hematoxylin and eosin stain. × 80.

FIG. 12. Vessels in the normal rabbit thyroid. Veins and venules are diffusely distributed in the thyroid parenchyma. Frozen section (250 μ), Pickworth stain. × 80.

FIG. 13. Distribution of auto-allergic lesions in the guinea pig adrenal. Inflammatory foci are present in the inner portion of the zona fasciculata and become most intense in the zona reticularis. Hematoxylin and eosin stain. × 40.

FIG. 14. Staining of the inner zone of the adrenal cortex in a guinea pig 20 minutes after the intravenous injection of Niagara sky blue 6B. Carnoy's fixative, paraffin section (16 μ). × 12.

FIG. 15. A higher power of the section shown in Figure 14 shows that the area of staining corresponds to the zona reticularis of the cortex. × 40.

FIG. 16. A radioautograph of an adrenal 18 hours after the intravenous injection of I^{131}-labeled human serum albumin. The label is seen only in the innermost zone of the cortex. × 6.

FIG. 17. Vessels of the normal guinea pig adrenal. Veins are largely limited to the zona reticularis and inner portion of the zona fasciculata. Frozen section (250 μ), Pickworth stain. × 36.

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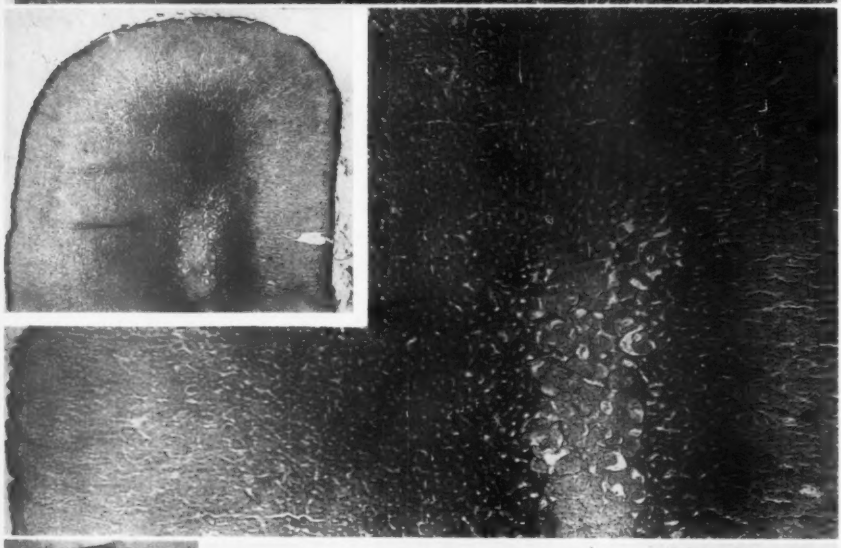

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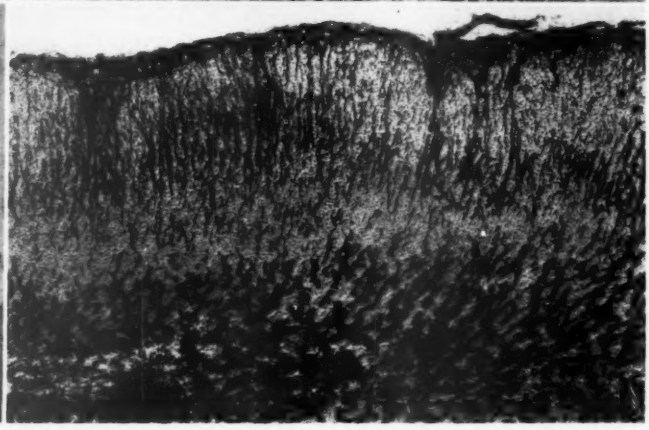

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A QUANTITATIVE STUDY OF GLOMERULAR ENLARGEMENT IN CHILDREN WITH TETRALOGY OF FALLOT

A Condition of Glomerular Enlargement Without an Increase in Renal Mass

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Pathologists have for some years noted enlargement of the glomeruli in the kidneys of children dying of cyanotic heart disease, but we are aware of only two publications on the subject in English.[1,2] Meessen and Litton[1] were the first to comment on this finding, but they made actual measurements in only two cases. We have confirmed and extended their observations by measurements on 29 cases of tetralogy of Fallot, and our findings form the basis of this report. Our emphasis has been on objective and quantitative confirmation of the glomerular enlargement, on any relationship it may bear to certain clinical findings, and on speculations as to the possible mechanisms involved.

Material and Methods

Thirty-one patients with tetralogy of Fallot were found in a review of the 5,484 consecutive necropsy examinations performed in the Department of Pathology, Washington University School of Medicine, from 1930 to 1958. Two were excluded from the study: one, a 12-year-old child with long-standing nephrosis in addition to the congenital cardiac anomaly; the other, a 57-year-old adult. The remaining 29 patients ranged in age at the time of death from 7 weeks to 21 years, with an average of 5.7 years. Table I gives a résumé of the significant clinical features.

Phlebotomies had been done in only 5 patients, with the removal of 100 cc. of blood in cases 17 and 21, unstated amounts in cases 24 and 28, and a total of 230 cc. in case 29. In several individuals there had been some clinical or anatomic evidence of renal abnormality. Case 12 had a horseshoe kidney and case 14 had a history of chronic renal tubular acidosis, for which no explanation was found anatomically. In case 16 a thrombus was found in the right renal artery at necropsy examination, and both cases 24 and 25 had albuminuria. Although some patients had enlargement of the liver at some time in the course, the full-blown picture of congestive heart failure was not a common occurrence. Cases 14, 25 and 29 had several episodes of failure which usually responded well to digitalis. Arterial oxygen saturation values were available in 11 cases. Nineteen of the 29 patients died either during an operative procedure or within 8 days thereafter.

Control cases were chosen at random from the necropsy files, and from these, 35 were selected as fulfilling the following criteria of "normality": the absence of chronic lung disease, cyanosis (except terminally) or clubbing; the presence of both kidneys without significant renal disease; the absence of congestive failure and of blood dyscrasia. These cases were chosen to cover the age range from newborn to 21 years;

TABLE I

TETRALOGY OF FALLOT

Case no.	Necropsy no.	Age at death (yr.)	Age at onset of cyanosis	Hgb (gm. %)	Rbc (millions/cmm.)	Terminal episode	Glomerular size *	Combined kidney wt. (gm.)
1	15743	.135	FB † on crying	15.1	4.73	Periodic apnea, excessive secretions	.93	29
2	16632	.333	FB	14.1	4.8	Found dead	1.01	55
3	14820	.333	3 mo.; slight (marked on exertion)	16.2	5.25	Died 3 hr. following Potts procedure in cardio-respiratory failure	.92	49
4	18428	.416	5 mo.; periodic	10.4 ‡	5.2	Died during thoracotomy	1.30	53
5	16188	.416	First wk.; on exertion	13.4	5.5	Died 2½ mo. post-op. with congestion and edema of lungs	1.57	40
6	16433	.50	FB, periodic; persistent at 4 mo.	17.7	7.5	Died 8 hr. following thoracotomy in cardio-respiratory failure	1.18	37
7	18302	.833	FB, periodic	13.3	7.0	Died 2 days following Brock procedure	1.35	62
8	18214	1.00	9 wk., on exertion; persistent at 3 mo.	16.5	7.9	Died 8 hr. following a second Potts procedure with partial occlusion of homograft by recent thrombus	1.22	74
9	17064	1.50	10 days	14.1	9.8	Thrombosis of internal carotid artery with recent encephalomalacia	1.69	70
10	17699	1.58	6 wk.	13.6	6.96	Died shortly following Blalock procedure	2.00	68
11	15220	1.58	2 mo., on exertion	16	6.72	Died during pulmonary valvulotomy	1.75	70
12	14230	2.0	FB, on exertion	17	9.2	Died 4 days after Potts procedure with bronchopneumonia	1.48	80

13	15869	2.5	FB; marked from 6 mo.	18 (dropped to 14 and	10.2 after Potts 7.0)	Potts procedure at 2¼ yr. Died fairly suddenly with fever	3.41	112
14	18053	2.5	2½ mo., episodic	11.1	6.5	Died during Brock procedure	2.63	74
15	16095	3.5	FB	21.0	8.0	Multiple focal cerebral hemorrhage	4.30	200
16	16081	4.0	FB	20.9	9.48	Thrombosis of Potts anastomosis done 8 days earlier	3.26	130
17	15719	4.0	4 mo.	21.4	10.0	Congestive failure and convulsions	3.20	130
18	17346	4.0	FB	17.3	6.0	Died 5 days following Potts procedure	2.02	90
19	13173	4.5	8 mo.	20.0	6.6	Died in pulmonary edema 1 day following Blalock procedure	2.32	75
20	17540	6	2 yr.	18.3	8.38	Died in shock following a valvulotomy	2.63	160
21	13694	7	FB	18.0	8.38	Died in pulmonary edema 1 day following Potts procedure	3.21	130
22	14611	10	3 yr.; mild, intermittent	16.2	6.15	Encephalomalacia 1 day following Blalock procedure	2.84	162
23	16676	10.5	Around 5 to 10 yr.	15.2	5.4	Died immediately following Brock procedure	2.38	160
24	16916	13	11 mo.	25.3	8.7	Died in failure 2 days following second operation (Blalock done at 12 yr.)	4.01	305
25	19139	13	6 mo.	14.7	5.4	Massive pulmonary infarcts from mural thrombi in right atrium, both ventricles	4.43	200
26	13495	16	3 yr.	23.0	8.0	Cardiac arrest during Potts procedure	4.86	220
27	12493	17	FB	27.0	8.6	Died 6 hr. following Potts procedure	6.76	290
28	12513	19	4 yr.	25.4	8.75	Multiple pulmonary emboli	7.66	310
29	16445	21	FB	26.8	9.0	Died following valvulotomy with cerebral edema	5.42	320

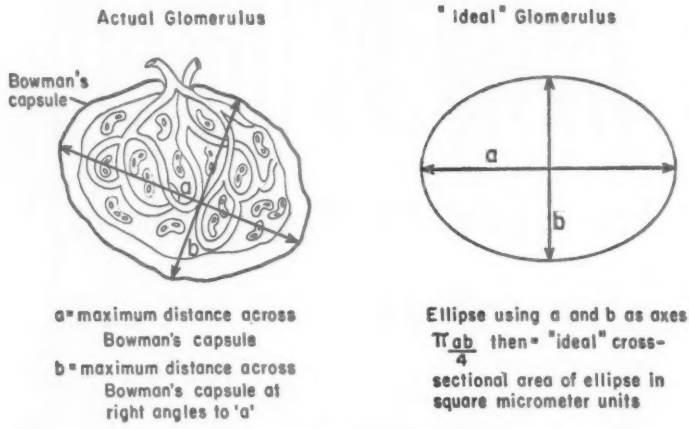
* Glomerular size given in square micrometer units.
† FB = from birth.
‡ This patient was thought to be anemic.

TABLE II

CAUSE OF DEATH IN 35 CHILDREN IN CONTROL SERIES

Acute infection with or without underlying disease	12
Malignant neoplasm	10
Disease of the central nervous system	7
Prematurity	2
Hypoxic nephrosis, early	1
Cirrhosis, hemorrhage	1
Toxic goiter	1
Sudden death, unexplained	1
Total:	35

at 2-month intervals from 2 months to 1 year, 6-month intervals from 1 to 2 years, and 1-year intervals thereafter, with an occasional omission. There were 17 boys and 18 girls. The terminal illness and cause of death are tabulated in Table II. Hemoglobin levels and frequently red blood cell values, as well, were known in all but 11 cases and were within the normal ranges. Sections from Zenker-fixed blocks of kidney tissue, embedded in paraffin, were stained with hematoxylin and eosin.


Actual Glomerulus
"Ideal" Glomerulus
Bowman's capsule
a
b
a
b
a = maximum distance across Bowman's capsule
b = maximum distance across Bowman's capsule at right angles to 'a'
Ellipse using a and b as axes
$\pi \frac{ab}{4}$ then = "ideal" cross-sectional area of ellipse in square micrometer units

TEXT-FIGURE 1. Method of measuring glomeruli and calculation of "ideal" cross-sectional area.

Technique of Measurement

A measure of average glomerular size was obtained in each case in the following manner: by means of an optical micrometer, 2 linear measurements were made for each of 100 glomeruli viewed at an enlargement of 430 times (Text-fig. 1). The first measurement "a" was the maximum distance across Bowman's capsule; the second, "b," the maximum distance across Bowman's capsule at right angles to the first measurement. These distances were measured and recorded in micrometer units, with the same optical measuring system for all cases. Each section was canvassed in a systematic manner from cortex to medulla, every glomerulus encountered being measured, no matter what the plane of section. Glomeruli obviously distorted by reason of artifacts of slide preparation were not measured.

From these measurements an "ideal" cross-sectional area was calculated by considering the two measurements, "a" and "b," for each glomerulus as the major and

minor axes of an ellipse, the area of which is given by $\frac{1}{4}\pi ab$ and recorded arbitrarily in square micrometer units. The average of 100 such measurements of "ideal" cross-sectional area was obtained in each case studied, and was taken as an expression of glomerular size.

An estimation of the difference between the "actual" average cross-sectional area of the glomeruli and the "ideal" average cross-sectional area was obtained by projecting the kidney sections from 4 patients with tetralogy of Fallot on a screen held at a fixed distance from the eyepiece to provide a glomerular image such that the cross-sectional areas were of a convenient order of magnitude. The 4 cases were chosen to represent the 2 extremes in values for average cross-sectional area and 2 intermediate values. The outlines of 100 glomeruli from each of these cases were traced on paper. Mensuration of the traced outlines, using a planimeter, provided the "actual" area values, and the "ideal" cross-sectional areas were calculated in the manner described.

Results

Accuracy of the "Ideal" Glomerular Area

The comparison of the "actual" and "ideal" average cross-sectional areas of the glomeruli in the 4 cases (Table III) showed good agreement between the planimeter and calculated values over the entire range of glomerular size under study. The error (± 3.6 per cent) was judged sufficiently small to permit the interpretation that the "ideal" average cross-sectional area in each case was representative of the "actual" average cross-sectional area as seen in the microsections. It should be emphasized here, however, that the parameter of glomerular area is expressed in arbitrary units and has value only in comparing one case with another.

Table III

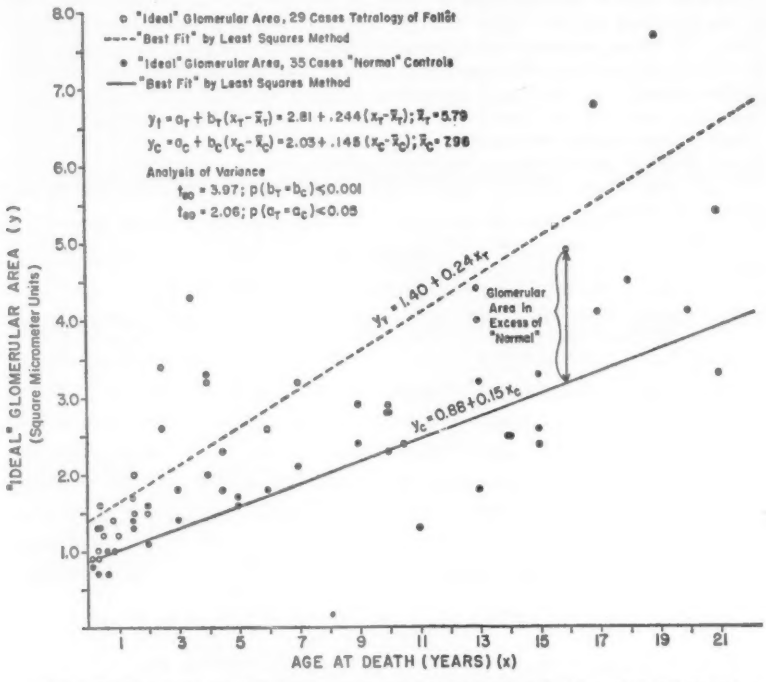
A COMPARISON OF THE "ACTUAL" AVERAGE CROSS-SECTIONAL AREA AND THE "IDEAL" AVERAGE CROSS-SECTIONAL AREA OF THE PROJECTED IMAGES OF 100 GLOMERULI IN 4 CASES OF TETRALOGY OF FALLOT

Case no.	Area (cm.²)		Per cent error: $\frac{\text{ideal} - \text{actual}}{\text{actual}} \times 100$
	"Actual" (planimeter values)	"Ideal" (calculated values)	
1	8.7	8.4	−3.5
20	21.3	21.8	+2.4
29	51.9	53.1	+2.3
28	67.6	70.0	+3.6

"Ideal" Glomerular Area of Tetralogy and Controls

The "ideal" average cross-sectional area of glomeruli of children with tetralogy of Fallot increased with increasing age at death. This is shown in Text-figure 2 in which these cross-sectional areas are plotted against the age at death. The regression line obtained by the method of least squares has a positive slope, and the probability that the regression coefficient is indistinguishable from a slope of zero is less than 0.001 (Table IV).[3] A similar dependence of glomerular area on age at death was

found for the "normal" control series (Text-fig. 2). The regression line obtained also had a positive regression coefficient that was highly significant (Table IV). In general, the values for glomerular area in chil-



TEXT-FIGURE 2. The "ideal" average cross-sectional areas of glomeruli plotted against age at death.

dren with tetralogy of Fallot tended to fall above the values for the control series throughout the entire age range. An analysis of variance (Table V) demonstrated that the probability of the slopes and the positions of the two regression lines being indistinguishable one from the other was less than 0.001 and less than 0.05 respectively. Thus, there was a significant difference between the average glomerular cross-sectional areas of the children with tetralogy of Fallot and of the children in the control series. At any given age at death, the tetralogy patients had kidneys with glomeruli almost twice the cross-sectional area of glomeruli of kidneys from children who died of other causes.

Histologic Observations in Patients with Tetralogy

The majority of the significant microscopic observations were restricted to the glomeruli; the immediate impression was one of glomeru-

lar enlargement, particularly in the older age groups (Figs. 1 to 8). We were also impressed by the appearance of hypercellularity of the glomerular tuft, elongation of the capillary loops without an increase in their number (Fig. 6) and congestion and ectasia of the glomerular capillaries (Fig. 8). In the glomerular tufts of some cases there appeared to be an increase in eosinophilic or hyaline intercellular substance (Figs. 4 and 6) similar to that described by Spear [2] and thought by him to involve the mesangium. The eosinophilic granular material which he also described was recognized in some glomeruli (Fig. 6). A favorable plane of section often showed dilated and congested arterioles (Fig. 8), a feature noted by Meessen and Litton [1] to be limited to the afferent limb. Vascular alterations other than generalized congestion were not observed in other portions of the kidney, nor was inflammation or periglomerular fibrosis a significant feature. Tubular changes were of a minor nature and were related in nearly all cases to postmortem effects.

TABLE IV

ANALYSIS OF THE SIGNIFICANCE OF THE REGRESSION COEFFICIENTS FOR RELATING THE AVERAGE CROSS-SECTIONAL GLOMERULAR AREA IN TETRALOGY AND CONTROL SERIES TO AGE AT DEATH

	Sum of squares	Degrees of freedom	Mean square	t	p
Tetralogy of Fallot					
Regression	67.1	1	67.1		
Error	18.85	27	0.698	9.81	<0.001
Total	85.95	28			
Controls					
Regression	27.61	1	27.61		
Error	5.45	33	0.165	12.9	<0.001
Total	33.06	34			

TABLE V

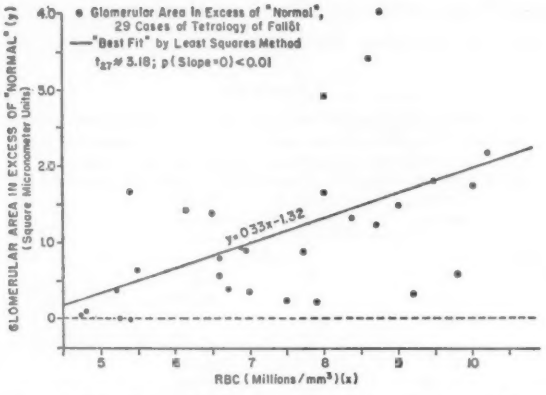
ANALYSIS OF VARIANCE OF "IDEAL" GLOMERULAR CROSS-SECTIONAL AREA IN CONTROL PATIENTS AND THOSE WITH TETRALOGY OF FALLOT

Item	Sum of squares	Degrees of freedom	Mean square	t_{∞}	p
Joint regression	88.33	1	88.33		
Difference between regressions	6.38	1	6.38	3.97	<0.001
Difference between means	1.722	1	1.722	2.06	<0.05
Error	24.30	60	0.405		
Total	120.732	63			

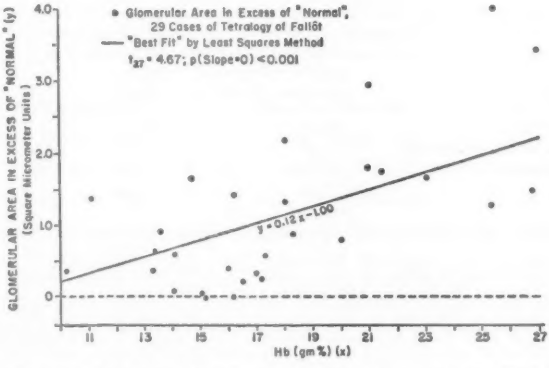
Relationship of Glomerular Cross-Sectional Area to Erythremia and Hemoglobin Values

In looking over the clinical data of the children with tetralogy of Fallot, it seemed that those with the highest red blood cell counts and

hemoglobin values, and presumably those with prolonged cyanosis, had the largest glomeruli. Since objective clinical information on the degree and duration of cyanosis was sparse, an attempt was made to correlate the hemoglobin values and severity of the erythremia with the degree of glomerular enlargement. The "ideal" average glomerular area for both the group with tetralogy and the controls was age-dependent. However, the hemoglobin values and red cell counts of the controls were all within a narrow range of normal values, i.e., not strictly age-dependent. Thus the usual method of covariant analysis could not be carried out.[8] There-


• Glomerular Area in Excess of "Normal",
29 Cases of Tetralogy of Fallôt
—"Best Fit" by Least Squares Method
t_{27}= 3.18; p(Slope=0)<0.01
GLOMERULAR AREA IN EXCESS OF "NORMAL" (y)
(Square Micrometer Units)
y=0.33x-1.32
RBC (Millions/mm³) (x)

TEXT-FIGURE 3. The glomerular areas in excess of normal for all tetralogy cases plotted against corresponding red cell counts.


• Glomerular Area in Excess of "Normal",
29 Cases of Tetralogy of Fallôt
—"Best Fit" by Least Squares Method
t_{27} = 4.67; p(Slope=0) <0.001
GLOMERULAR AREA IN EXCESS OF "NORMAL" (y)
(Square Micrometer Units)
y = 0.12 x - 1.00
Hb (gm%) (x)

TEXT-FIGURE 4. The glomerular areas in excess of normal for all tetralogy cases plotted against corresponding hemoglobin values.

fore, a newly contrived parameter of glomerular area was utilized for these correlations; this minimized the age dependence of glomerular size. The difference between the value of the "ideal" average cross-sectional

area for each case of tetralogy of Fallot and a value of glomerular area at the appropriate age of death, as given by the regression line for the con-

TABLE VI

ANALYSIS OF THE SIGNIFICANCE OF THE REGRESSION COEFFICIENTS FOR RELATING AVERAGE "GLOMERULAR AREA IN EXCESS OF NORMAL" TO ERYTHREMIA AND HEMOGLOBIN VALUES

	Sum of squares	Degrees of freedom	Mean square	t	p
Erythremia					
Regression	8.12	1	8.12		
Error	21.64	27	.801	3.18	< 0.01
Total	29.76	28			
Hemoglobin values					
Regression	13.31	1	13.31		
Error	16.45	27	.609	4.67	< 0.001
Total	29.76	28			

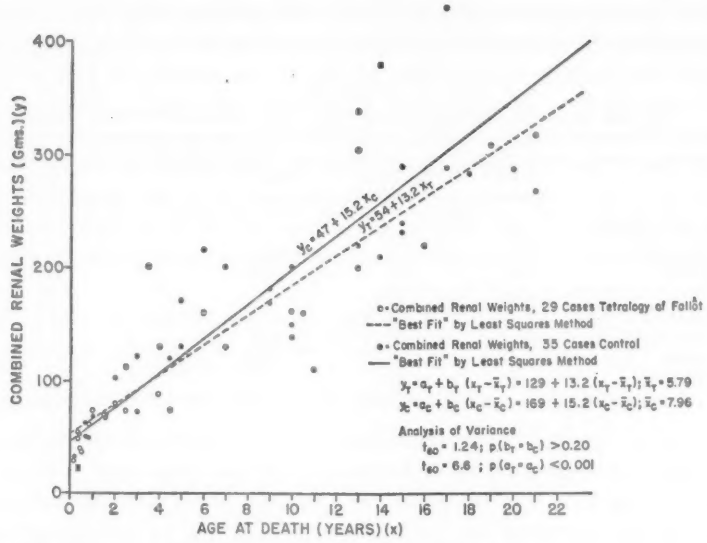
trol series, was calculated and termed the "glomerular area in excess of normal." A diagrammatic representation of this parameter is given in Text-figure 2. These values for all tetralogy cases were then plotted against the corresponding red cell counts (Text-fig. 3) and hemoglobin values (Text-fig. 4). The regression line for each plot has a positive slope and is significantly different from zero (Table VI). Thus, the greater the severity of erythremia and the higher the hemoglobin values (and presumably the higher the hematocrit and the greater the severity or duration of cyanosis), the greater was the increase of glomerular size over the normal.

Renal Mass

One last observation of great interest was that the total renal mass of children with tetralogy of Fallot was found to be somewhat smaller than that of the controls. In Text-figure 5 the combined weight of both kidneys in cases of tetralogy and controls is plotted against the age at death. An analysis of variance (Table VII) of the two regression lines demonstrated no significant difference between the regression coefficients, but it did indicate that the position of the regression line for the controls was significantly higher than the line for the patients with tetralogy. Hence, the pathogenetic factors responsible for an increase in the size of the glomerulus did not give rise to a detectable increase in renal mass. In other words, the tubular portion of the nephron did not increase in size.

DISCUSSION

The results of this study provide objective and quantitative confirmation of glomerular enlargement in cyanotic congenital heart disease as first reported in 1953 by Meessen and Litton.[1] Their observations were


COMBINED RENAL WEIGHTS (Gms.)(y)
AGE AT DEATH (YEARS)(x)
400
300
200
100
0
0 2 4 6 8 10 12 14 16 18 20 22
$y_c = 47 + 15.2\,x_c$
$y_T = 54 + 13.2\,x_T$
c = Combined Renal Weights, 29 Cases Tetralogy of Fallot
"Best Fit" by Least Squares Method
c = Combined Renal Weights, 35 Cases Control
"Best Fit" by Least Squares Method
$y_T = a_T + b_T\,(x_T - \bar{x}_T) = 129 + 13.2\,(x_T - \bar{x}_T);\ \bar{x}_T = 5.79$
$y_C = a_C + b_C\,(x_C - \bar{x}_C) = 169 + 15.2\,(x_C - \bar{x}_C);\ \bar{x}_C = 7.96$
Analysis of Variance
$t_{60} = 1.24;\ p(b_T = b_C) > 0.20$
$t_{60} = 6.6\ ;\ p(a_T = a_C) < 0.001$

TEXT-FIGURE 5. Kidney weights plotted against age at death.

included in a report of the pathologic alterations occurring in 28 patients with congenital heart disease; 24 of these had tetralogy of Fallot, 1 had *cor biloculare* with a hyperplastic pulmonary artery, 2 had pul-

TABLE VII

ANALYSIS OF VARIANCE OF COMBINED KIDNEY WEIGHTS IN CHILDREN WITH TETRALOGY OF FALLOT AND CONTROLS

Item	Sum of squares	Degrees of freedom	Mean square	t_{60}	p
Joint regression	490,779	1	490,779		
Difference between regressions	2,455	1	2,455	1.24	>0.20
Difference between means	68,644	1	68,644	6.6	<0.001
Error	95,047	60	1,548		
Total	656,925	63			

monary stenosis associated with a patent ductus, and 1 had an isolated patent ductus. These cases ranged in age from 3 months to 28 years; all had elevated erythrocyte counts from 5.4 to 9.2 million per cu. mm. with an average of 7.7 million per cu. mm., and all but 4 were cyanotic. The authors concluded from their visual impression of glomerular size and an evaluation of the clinical data that a striking correlation existed between the age at death and degree of cyanosis on the one hand and the degree of glomerular enlargement on the other.

In a recent paper Spear[2] has also commented upon glomerular en-

largement in 16 of 288 cases of cyanotic congenital heart disease. Particular attention was drawn to these cases because of the distinctive appearance of the glomeruli, which were characterized by glomerular congestion, hypercellularity and intercapillary deposition of a granular or fibrillar eosinophilic material.

Glomerular enlargement has been produced in experimental animals under similar conditions of chronic hypoxia and erythremia. Highman and Altland[4] produced a profound elevation (100 per cent) in hematocrit and hemoglobin values by exposing 14-day-old rats to simulated altitudes of 25,000 feet for 4 hours daily for periods up to 1 year. Glomerular enlargement, as measured by the number of rats having average glomerular diameters in excess of 130 μ, was found in 49 of 95 exposed rats and in only 4 of the unexposed controls. In a second experiment,[5] using a simulated altitude of 18,000 feet, the same workers found erythrocyte counts in the exposed rats increased 56 per cent over the control values. The glomeruli in these animals were 10 to 25 per cent larger in average diameter than the glomeruli of unexposed controls. The enlargement was interpreted by them to be a result of renal capillary dilatation to accommodate an increased blood volume, and was reflected in the marked capillary engorgement found in these kidneys.

Kindred,[6] however, was unable to demonstrate any glomerular enlargement on histologic examination of the kidneys of adult male rats exposed by Silvette[7] to a simulated altitude of 15,000 feet for 3 hours daily for a period of 27 days. The kidneys in a group of rats exposed to a simulated altitude of 25,000 feet under similar experimental conditions showed early ischemic necrosis of tubules in scattered areas in the cortex, but the glomerular size in this group was not determined. The failure to find glomerular enlargement in the 15,000 feet group may have been due to the use of adult animals or to the relatively short exposure periods.

Enlarged glomeruli have also been found as part of the compensatory hypertrophy that occurs in the remaining kidney following unilateral nephrectomy. Saphir[8] reported that the glomerular enlargement seen in unilateral nephrectomized rabbits was primarily due to an increase in the size of the cells of the capsule and glomerular tuft. Similar findings were reported by Rollason[9] for the rat. That there was no increase in the number of nephrons was shown by Moore[10] in the case of compensatory hypertrophy and by Meessen and Litton[1] in the case of *morbus caeruleus*. However, the similarity in anatomic features in kidneys with compensatory hypertrophy and those of cyanotic congenital heart disease ends here. There is general agreement that in unilateral nephrectomy all components of the nephrons of the remaining kidney increase in size. The net result is an increase in renal mass, a finding not observed in

this study with cyanotic heart disease. Mention should be made here that Kindred [6] was able to show a statistically significant increase in kidney weights in rats exposed to simulated altitudes of 15,000 to 25,000 feet. The increased renal mass was not correlated with the degree of erythremia in these animals (the group kept at 15,000 feet showed no increase in erythrocyte count; the group at 25,000 feet showed an increase of 52 per cent over the control values) but was correlated with the degree of glomerular hyperemia. The latter finding and the intense hyperemia observed in these kidneys suggested to Kindred that the entire observed increased renal mass could be accounted for by the additional blood present.

In searching for a possible explanation for the glomerular enlargement, Meessen and Litton [1] observed that the capillaries of the glomerular tuft and the vasa afferentia, but usually not the vasa efferentia, were widened. They reasoned that the glomerular enlargement was a reflection of the ectasia of the capillaries, and that this in turn resulted from a dilatation of the afferent arteriole in response to the chronic hypoxia.

In investigating the renal function and hemodynamics of 19 patients with congenital cyanotic heart disease, Scott and Elliott [11] found the corrected filtration fraction to be elevated, the effective renal plasma flow reduced and the filtration rate unchanged when compared with the appropriate normal values. Functionally, then, these glomeruli provided the tubules with a normal volume of filtrate per unit time by filtering an increased proportion of the water from the available plasma even in the face of a reduced plasma flow. These authors cited the opinion of Smith [12] in this situation; namely, that low renal plasma flow in the presence of an increased filtration fraction indicates efferent arteriolar constriction. They suggested that the high blood viscosity associated with the high hematocrit values in these cyanotic subjects was increased to an extreme degree in the region of the efferent arteriole and could, therefore, be responsible for an increase in post-glomerular resistance. Similarly, it is conceivable that an increase in post-glomerular resistance secondary to high blood viscosity might also be responsible in part for the ectasia of glomerular capillaries and glomerular enlargement. The observation in this study of a correlation between glomerular size and erythrocyte counts and hemoglobin values is consistent with such a view; however, the exact role of blood viscosity and chronic hypoxia in the pathogenesis of glomerular enlargement must await further studies.

It is interesting to speculate on the role of increased glomerular size in the alterations of renal physiology in children with cyanotic congenital heart disease. As a first approximation, it seems reasonable to assume that the total filtration area of the kidney and the average

glomerular cross-sectional area both are functions of the square of the average glomerular radius and that glomerular volume varies as the 3/2 power of the average cross-sectional area. It would follow then that the total surface area for filtration in children with tetralogy of Fallot is almost twice and the glomerular volume almost 3 times that of the normal controls. It is as though the kidneys of the affected children had the filtration area of 4 and the glomerular volume of 6 normal kidneys. This would mean that even though each milliliter of blood delivered to the kidney had a smaller amount of plasma from which to make filtrate because of the high hematocrit, the total renal blood flow could be increased so as to effect a normal renal plasma flow. Consequently there would be a normal filtration rate, without necessitating any adjustment of hydrostatic pressure within the glomerulus. It would appear, however, from the work of Scott and Elliott [11] that the effective renal plasma flow is significantly decreased even in the face of an increased total renal blood flow. Hence, assuming that formation of filtrate occurs under conditions of equilibrium, glomerular enlargement alone cannot maintain the normal filtration rate in this situation. An increase in filtration fraction is necessary to accomplish this and implies some adjustments of hydrostatic pressure in the glomerulus. This line of thinking provides additional theoretical reasons in support of the view that the increased blood viscosity associated with erythremia is an important factor in the physiologic adjustments of the kidney in this condition and that the glomerular enlargement follows as a consequence. However, if filtrate formation is a nonequilibrium process, then the increased filtration fraction could be a result of enlarged filtration area.

The lack of any increase in renal mass is more easily understood. The maximal excretory capacity of kidneys of subjects with cyanotic congenital heart disease is unchanged, as evidenced by the normal TmPAH values reported by Scott and Elliott.[11] It seems reasonable to anticipate that the maintenance of a normal glomerular filtration rate necessitates no increase in the work of the tubules in making urine. Hence, there is not the stimulus for hypertrophy that operates in the remaining kidney in unilateral nephrectomy. Congenital cyanotic heart disease is the only human condition thus far in which there is quantitative evidence of an increase in glomerular filtration area and glomerular volume without associated renal tubular hypertrophy.

Summary

Measurements were made of the average glomerular size in 29 cases of tetralogy of Fallot and 35 control cases, and a significant enlargement was noted in the former group. A positive relationship was found be-

tween the severity of erythremia and height of hemoglobin values on the one hand and the glomerular size on the other. Interestingly enough, the combined renal weights were not increased, indicating that there was not an associated increase in size of the tubular portion of the nephron. Reports related to this subject are reviewed, and the possible mechanisms involved are discussed.

References

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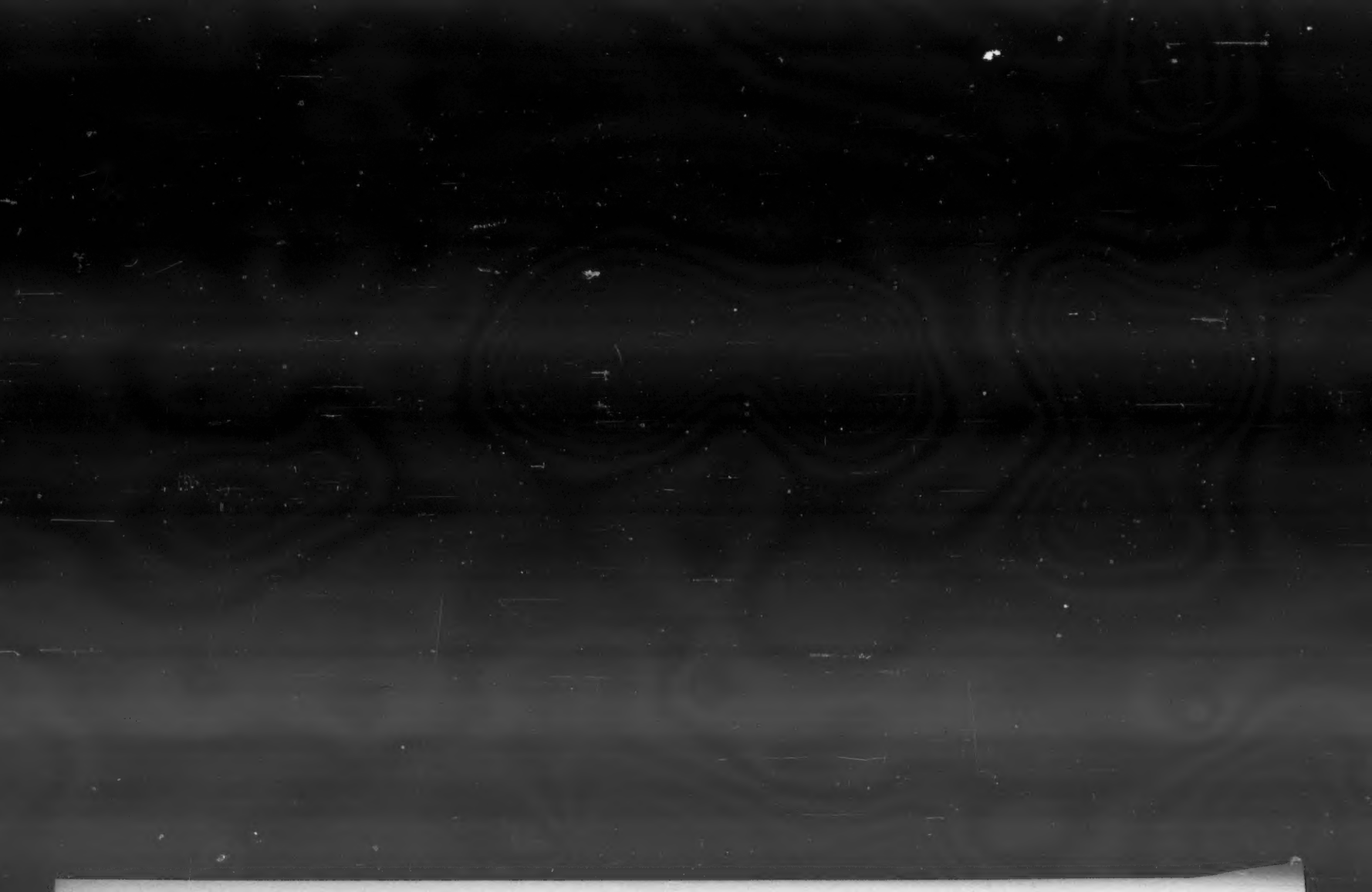
[*Illustrations follow*]

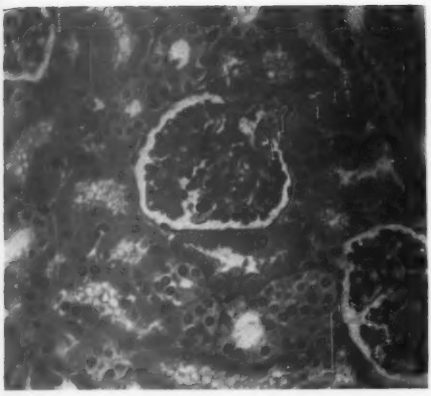
Legends for Figures

Photographs were prepared from sections stained with hematoxylin and eosin.

Figs. 1 and 3. A midsagittal section of a representative glomerulus from the kidney of a child who died with hydrocephalus at the age of 6 months (Fig. 1), and a child who died of meningitis at 3 years of age (Fig. 3). × 100.

Figs. 2 and 4. A comparable view of a glomerulus from the kidney of a child with tetralogy of Fallot who died at 5 months of age (Fig. 2), and a child with tetralogy of Fallot who died at the age of 3.5 years (Fig. 4). × 100.





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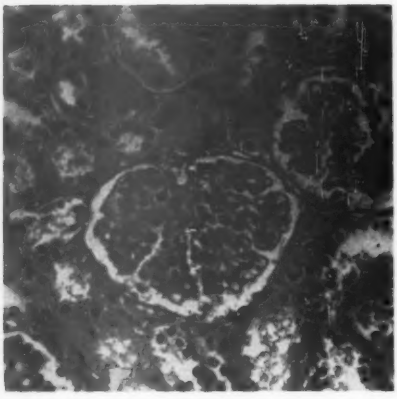

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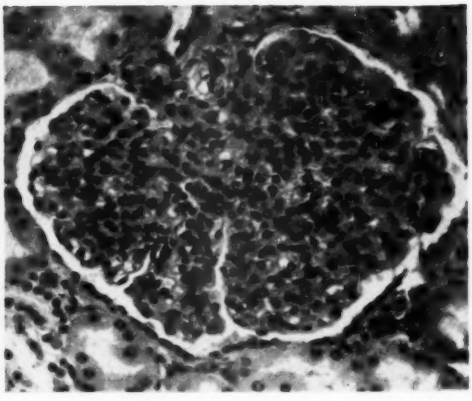

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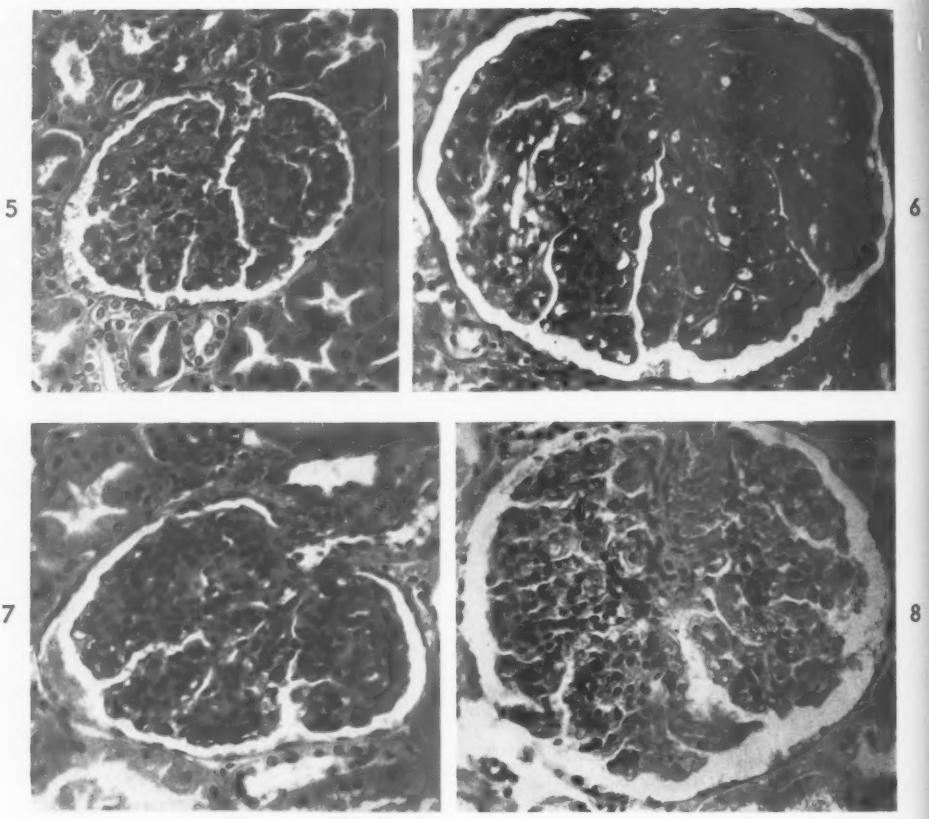

FIGS. 5 and 7. A midsagittal section of a representative glomerulus from the kidney of a child dying of a malignant tumor at the age of 12 years (Fig. 5), and a girl who died of postnecrotic cirrhosis at the age of 18 years (Fig. 7). × 100.

FIGS. 6 and 8. A comparable midsagittal section of a representative glomerulus in a 12-year-old child who died with tetralogy of Fallot (Fig. 6), and a 19-year-old girl who had tetralogy of Fallot and died with multiple pulmonary emboli (Fig. 8). × 100.

THE EFFECT OF RENAL HYPERTENSION ON CHOLESTEROL ATHEROSCLEROSIS IN CORTISONE-TREATED RABBITS

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Repeated observations have indicated that the administration of cortisone produces an elevation of all lipid fractions in the blood of man as well as in some experimental animals, particularly the rabbit. Because of this, it has been suggested that prolonged cortisone administration in man may augment the atherosclerotic process.[1,2] Etheridge and Hoch-Ligeti[3] have reported an increase in the degree of atherosclerosis in young persons treated with cortisone although confirmation of this observation or other evidence implicating this agent with the development of atherosclerosis in humans has not been presented. Indeed, despite the elevation of serum lipids following cortisone administration in both normal and cholesterol-fed (c-f) rabbits, several investigators have observed that aortic cholesterol atherosclerosis in this species is actually inhibited.[4,5] The mechanism accounting for this inhibitory effect has not been conclusively elucidated. Gordon, Kobernick, McMillan and Duff[6] suggested that several factors might be responsible. They observed the hypercholesteremia in cortisone-treated, cholesterol-fed (c-t, c-f) rabbits to be significantly lower than that observed in untreated c-f animals although other lipid fractions were more elevated in the former. That the lower cholesterol values might account for the milder degree of atherosclerosis was in keeping with the recognition that generally the degree of atherosclerosis in the c-f rabbit may be correlated with the degree of hypercholesteremia. Lower cholesterol:phospholipid ratios accompanying the relatively lower level of cholesterol in the c-t, c-f rabbits also appeared as a factor in preventing atherosclerosis in these animals, being analogous to that observed in c-f rabbits with alloxan diabetes[7] or those treated with detergents.[8] Although Stumpf and Wilens[9] emphasized the significance of relatively low cholesterol levels in c-t, c-f rabbits in inhibiting the atherosclerotic process, they did not find altered cholesterol:phospholipid ratios in their experiments. Their observation in this respect was in agreement with that recorded by Oppenheim and Bruger.[4] Gordon and co-workers[6] also indicated that the reduction of

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atherogenic lipoproteins during prolonged cortisone administration, as noted by Pierce and Bloom,[10] might also play a role in the inhibition of the atherosclerosis observed. Adlersberg and associates,[5,11] in several communications, have considered that a decrease in tissue permeability associated with cortisone administration may provide an explanation for these experimental findings. The evidence in support of their contention consisted principally of the divergent effects of cortisone and hyaluronidase on the atherosclerotic process in c-f rabbits as noted in their experiments, as well as in those of Seifter and colleagues.[12]

Recent experiments performed in our laboratory have indicated that the greater degree of atherosclerosis in hypertensive c-f rabbits than in normotensive c-f animals may, at least in part, be related to an apparent increase of aortic ground substance observed in the rabbits with renal hypertension.[13] A similar aortic alteration has also been noted in hypertensive, alloxan-treated, c-f rabbits in which the inhibitory effect of diabetes on cholesterol atherosclerosis was superseded by the aggravating effect of hypertension since such animals exhibited more severe atherosclerosis than c-f, normotensive, nondiabetic controls.[14] Because of this effect of hypertension on aortic ground substance and its possible relationship to the atherosclerotic process, it was considered worth while to investigate the effect of hypertension on c-t, c-f rabbits. The results obtained provide additional information concerning the mechanism of cortisone inhibition of cholesterol atherosclerosis as well as emphasizing the augmenting effect of hypertension on this process.

Material and Methods

Seventy-seven adult male and female albino rabbits weighing approximately 2 kg. each were divided into 7 groups. Group I consisted of 12 rabbits maintained on the stock laboratory diet. These received intramuscular injections of cortisone acetate (Cortone,® Merck Company) according to the following schedule: 5 mg. per day during the first week; 10 mg. per day during the third, and 15 mg. per day during the fifth, seventh and ninth weeks of the experiment. Group II comprised 10 animals that received the stock laboratory diet containing 2 per cent cholesterol. Group III consisted of 12 cholesterol-fed (c-f) rabbits that received cortisone. Group IV was composed of 15 cholesterol-fed, cortisone-treated (c-f, c-t) animals in which the induction of hypertension was attempted by the method of Page,[15] except that both unilateral nephrectomy and cellophane enclosure of the contralateral kidney were performed in one stage. Group V consisted of 10 c-f rabbits in which hypertension was induced. A similar number comprised group VI; these were subjected to the Page procedure and cortisone treatment but were maintained on the stock laboratory diet. There were 8 animals in group VII. These were maintained on the stock laboratory diet and subjected to the Page procedure only.

All animals were weighed at the beginning of the experiment and at weekly intervals and were sacrificed by the intravenous administration of a lethal dose of Nembutal® at 60 to 65 days following operation and/or the administration of cholesterol and cortisone.

Estimation of Blood Pressure

Blood pressure was determined in all animals at weekly intervals by the ear-capsule technique of Grant and Rothschild.[18] Animals were rested for 5 minutes in an isolated room before the estimation of blood pressure, and the average of 3 successive determinations was recorded. Control blood pressures were obtained for each animal prior to any experimental procedure by calculating the average of 3 consecutive daily determinations.

Biochemical Studies

Blood was obtained prior to sacrifice by lacerating a marginal ear vein. Total blood cholesterol and esters were determined according to the method of Schoenheimer and Sperry; [17] total lipid by the method of de la Huerga, Yesinick and Popper; [18] lipid phosphorus as described by Youngburg and Youngburg; [19] and nonprotein nitrogen (N.P.N.) by the standard Kjeldahl technique. In some animals of each group, blood sugar determinations were made according to the method of Nelson.[20]

Anatomic Studies

At the time of sacrifice the aorta was removed quickly, opened and stripped of periadventitial adipose and connective tissue. The degree of atherosclerosis was determined arbitrarily by computing the average of the grades of atherosclerosis in the arch, and the thoracic and abdominal segments (*vide infra*) as described previously.[13] One half of the aorta was prepared for manometric studies, the results of which will be published in another communication.[21] The other half was divided into segments consisting of the arch (from aortic valve to a point one cm. distal to the origin of the great vessels), the thoracic aorta (from the distal end of the arch to the level of the diaphragm) and the abdominal aorta (the distal end of the thoracic segment to the bifurcation of the iliacs). The heart, liver and adrenals were cleaned and weighed.

Blocks of heart, lung, pancreas, brain, spleen, kidney, gonad, adrenal, skeletal muscle, thyroid, bone marrow and aorta were fixed in 10 per cent neutral formalin. Some of the aorta was also fixed in absolute alcohol. Paraffin sections were processed in the usual manner, and sections from all tissues were stained with hematoxylin and eosin. In addition, sections of aorta were stained with thionine (pH 4, 1:10,000) with and without antecedent treatment with testicular hyaluronidase, Alcian blue, and by the Rinehart-Abul-Haj, Wilder reticulum, Verhoeff-van Gieson, and Masson trichrome procedures.

The degree of metachromasia encountered in the intima, and in the inner and outer portions of the media was subjectively graded. This provided an index for this alteration in the various layers as well as in the entire aorta examined.

Results

General Effects

A total of 21 rabbits that received cortisone, including animals maintained on the stock diet with or without cholesterol supplement and/or hypertension, succumbed prior to the conclusion of the experimental period. Ten animals subjected to the induction of hypertension or supplementary cholesterol feeding also succumbed. Only animals which survived the experimental period were considered for analysis. Although actual measurements of food intake were not performed, observation indicated that all animals treated with cortisone ingested slightly less

TABLE I

EFFECT OF CORTISONE AND/OR HYPERTENSION ON BODY WEIGHT, BLOOD PRESSURE, NONPROTEIN NITROGEN AND SERUM LIPIDS OF CHOLESTEROL-FED AND NON-CHOLESTEROL-FED RABBITS

	Group	No.	Mean body wt. (kg.) Begin.	End	Diff.	Mean blood pressure * (mm. Hg)	Mean cholesterol/ester (mg. %)	Mean lipid phosphorus (mg. %)	Mean total lipid (mg. %)	Mean N.P.N. (mg. %)
I	Cortisone	6	2.2	2.1	−0.1	84	290/106	10	340	33
II	Cholesterol	10	2.2	2.9	+0.7	78	1648/1100	22	1850	40
III	Cholesterol and cortisone	7	2.3	2.5	+0.2	84	1115/850	32	1950	25
IV	Cortisone, cholesterol and hypertension	5	2.3	2.3	0	120	1266/1125	40	2360	38
V	Cholesterol and hypertension	4	2.2	2.3	+0.1	118	1700/828	19	2217	46
VI	Cortisone and hypertension	4	2.3	2.1	−0.2	114	300/139	9	469	49
VII	Hypertension	4	2.4	2.4	0	116	101/63	4	207	40

* Average throughout experimental period.

P values:

Blood pressure: I, II, III and IV, V, VI and VII, < 0.01

Total cholesterol: II, V and III, IV, $< .01$; I, VI and VII, $< .05$

Total lipid: II, III, IV, V and I, VI, VII, $< .01$

Lipid phosphorus: I, II, V, VI, VII and III, IV, $< .01$; I, VI and VII, $< .01$

food than those untreated. All animals failed to exhibit a significant gain or loss of body weight throughout the experimental period (Table I). The slight gain observed in normotensive c-f rabbits was less than that observed in normal animals maintained on the stock ration in our laboratory. The latter exhibited an average weight increase of 1.2 kg. during a comparable period. The difference observed in mean body weight of normotensive, untreated c-f animals and those treated with cortisone and maintained on the stock diet was of questionable significance ($P < .05$).

Hypertension was present only in animals subjected to the Page procedure and appeared in those in which its induction was successful 1 to 2 weeks following operation. The elevations of blood pressure observed in these animals were significantly greater than control values as well as those observed in unoperated animals of other groups ($P < .01$).

Biochemical Findings

As indicated in Table I a moderate but comparable elevation of all lipid fractions examined was apparent in normotensive and hypertensive c-t rabbits maintained on the stock laboratory diet. Similarly, no significant difference was apparent in the marked elevations of serum lipids in c-f, normotensive and hypertensive animals not treated with cortisone. However, the administration of cortisone to c-f rabbits resulted in less hypercholesteremia and greater total lipid and lipid phosphorus values than observed in untreated c-f animals. As a consequence, cholesterol: phospholipid ratios were notably depressed in the former. No striking difference in the ratio of total to esterified cholesterol was noted in c-t and untreated c-f animals. Hypertension had no apparent effect on differences in serum lipids observed in c-t and untreated c-f animals.

No significant elevations of N.P.N. or blood sugar were observed in any group investigated.

Effect of Cortisone and/or Renal Hypertension on Aortic Atherosclerosis

Cortisone administration inhibited the degree of aortic atherosclerosis in cholesterol-fed rabbits (Table II; Text-fig. 1). However, the degree of aortic atherosclerosis in c-t, hypertensive c-f rabbits was similar to that observed in normotensive c-f controls, although less than that in untreated c-f hypertensive animals. There was no consistent correlation between the level of blood pressure and degree of atherosclerosis in individual animals. Aortic atherosclerosis was not evident in normotensive or hypertensive c-t rabbits maintained on the cholesterol-free diet.

The microscopic appearance of the aortic lesions in sections stained

TABLE II

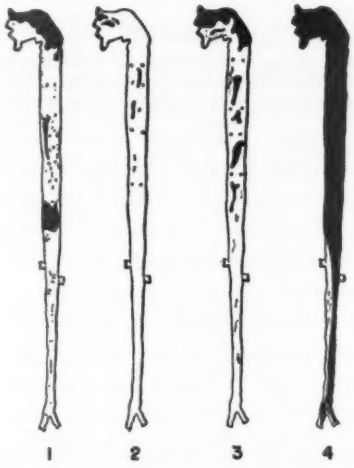
DEGREE OF ATHEROSCLEROSIS AND METACHROMASIA OF AORTAS IN CORTISONE-TREATED AND UNTREATED, NORMOTENSIVE AND HYPERTENSIVE CHOLESTEROL-FED AND NON-CHOLESTEROL-FED RABBITS

	Group	No.	Atherosclerosis				Metachromatic index †
			Arch	Thoracic	Abdominal	Total Av.*	
I	Cortisone		0	0	0	0 ‡	0.09 ‡
II	Cholesterol	0	1+	1+	0	0.7	0.16
		1	3+	2+	±	1.8	0.18
		3	2+	1+	0	1.0	0.86
		4	1+	±	0	0.5	0.20
		5	2+	1+	±	1.2	0.26
		30	3+	2+	0		0.46
		40	2+	1+	+	1.2	0.60
		70	1+	±	0	0.5	0.23
		80	2+	±	0	0.8	0.40
		100	1+	1+	0	0.7	0.36
						1.3 ‡	0.53 ‡
III	Cortisone and cholesterol	5	±	±	0	0.3	0.06
		6	1+	±	0	0.5	0.53
		7	2+	1+	0	1.0	0
		13	0	0	0	0	0.06
		15	±	0	0	0.2	0.36
		21	±	±	0	0.3	0.43
		23	0	0	0	0	0.06
						0.3 ‡	0.21 ‡
IV	Cortisone, cholesterol and hypertension	10	1+	1+	0	0.7	0.46
		55	3+	1+	±	1.5	0.46
		62	2+	±	0	0.8	0.36
		73	3+	2+	±	1.8	0.46
		110	1+	±	0	0.5	0.73
						1.1 ‡	0.49 ‡
V	Cholesterol and hypertension	75	3+	2+	1+	2.0	1.33
		76	3+	2+	±	1.8	1.13
		77	3+	1+	0	1.3	1.30
		78	3+	2+	2+	2.3	0.80
						1.9 ‡	1.14 ‡
VI	Cortisone and hypertension		0	0	0	0 ‡	0.24 ‡
VII	Hypertension		0	0	0	0 ‡	1.20 ‡

* Average of all segments.
† Average of intima and media of all segments.
‡ Average of entire group.

with hematoxylin and eosin was qualitatively similar in all c-f animals. However, the presence of aortic ground substance was notably different in the various groups. As indicated in Table II, metachromasia was greatest in aortas from hypertensive rabbits not receiving cortisone. This reaction was most intense in the intima-medial zone. Metachromatic substance in the aortas from hypertensive c-t, c-f rabbits was

comparable to that observed in c-f controls which exhibited a greater quantity of this material than normotensive c-t animals maintained on the stock laboratory diet or that with cholesterol supplement (Fig. 1). The Alcian blue and Rinehart-Abul-Haj reactions paralleled the findings noted with thionine-induced metachromasia. The arch exhibited



TEXT-FIGURE 1. Schematic representation of degrees of aortic atherosclerosis in cholesterol-fed rabbits. (1) Untreated, normotensive. (2) Cortisone-treated, normotensive. (3) Cortisone-treated, hypertensive. (4) Untreated, hypertensive.

the greatest degree of metachromasia and the abdominal segment the least. The variations in aortic mucopolysaccharide were not limited to areas with atherosclerotic change. All reactions for acid mucopolysaccharide were in large part resistant to prior treatment with testicular hyaluronidase. The intimal plaques encountered in untreated, hypertensive c-f rabbits contained more fibrosis than was observed in the lesions of normotensive c-f animals. No significant quantitative or qualitative differences in the reticulin pattern or elastic fibers were observed in c-f rabbits. Disruption of the latter was apparent only at the intima-medial zones subtending atherosclerotic plaques.

Other Vessels

Atherosclerosis of pulmonary, coronary and other systemic vessels was variable in c-f animals. However, the impression was gained that atherosclerotic alteration of these vessels was less in cortisone-treated than in untreated animals.

Viscera

The weights of the heart, liver and adrenals from animals of the various experimental groups are tabulated in Table III. Hypertension was accompanied by a significant increase in cardiac weight. The weights of

TABLE III

EFFECT OF CORTISONE AND/OR HYPERTENSION ON MEAN WEIGHT OF HEART, LIVER AND ADRENALS OF CHOLESTEROL-FED AND NON-CHOLESTEROL FED RABBITS

	Group	Heart (gm.)	Liver (gm.)	Adrenals (mg.)
I	Cortisone	7.5	86	124
II	Cholesterol	7.0	118	700
III	Cortisone and cholesterol	7.1	112	298
IV	Cortisone, cholesterol and hypertension	8.8	85	315
V	Cholesterol and hypertension	9.3	76	830
VI	Cortisone and hypertension	8.6	83	125
VII	Hypertension	9.6	76	305

P values:
Heart: I, II, III, and IV, VI, VII, $< .05$
Liver: II, III, and I, IV, V, VI, VII, $< .05$
Adrenals: I, VI and II, III, IV, V, VII, $< .01$
II, V and III, IV, $< .01$

the adrenals in animals receiving cortisone were significantly less than those observed in animals from comparable groups not receiving this agent. No significant difference in hepatic weights or lipid deposition could be discerned in c-f animals of any group except that the weight of the liver and the degree of hepatic lipidosis were strikingly less in untreated, hypertensive c-f animals than noted in other animals receiving cholesterol. Lipid deposits in the spleen were quantitatively less in c-t, c-f and hypertensive c-f rabbits than in spleens from untreated c-f animals. On the other hand, fatty change and interstitial accumulations of lipid-laden macrophages were more evident in normotensive and hypertensive c-t, c-f rabbits than in untreated c-f controls. The glomeruli from c-t animals failed to reveal significant morphologic alteration although a rare glomerulus in one animal that received cholesterol in addition to cortisone contained a large lipid deposit within a capillary loop.

Sex

Although the number of animals of each sex in any particular group was small, no significant qualitative or quantitative differences in the macroscopic or microscopic appearance of the lesions described could be discerned in the two sexes.

Discussion

The results of this study confirm previous observations indicating that cortisone administration inhibits the severity of atherosclerosis in cholesterol-fed (c-f) rabbits. However, unlike the observations of Wang, Schaefer and Adlersberg,[5] we have observed similar degrees of hepatic lipidosis. Only the degree of lipid deposition in the spleen and pulmonary and systemic vessels was significantly less in the c-t, c-f animals. However, fatty change of the kidney and interstitial deposits in this organ were notably more severe in these animals than in untreated c-f rabbits. These findings differ from those observed in diabetic rabbits fed cholesterol, in which all visceral deposits appeared less than those observed in untreated c-f rabbits.[13] We have no explanation for the divergent results in this study and that of Wang and co-workers,[5] or the situation encountered in diabetic rabbits. It appears notable, however, that Gordon and co-workers[6] and Stumpf and Wilens[9] in their thorough studies of the effect of cortisone on cholesterol atherosclerosis in this species failed to mention variations in visceral lipidosis. The significant decrease of adrenal weights noted in c-t rabbits is in keeping with the well recognized effect of cortisone on these organs and may be utilized as an indicator of the effectiveness of cortisone treatment. Although cortisone has been observed to produce renal glomerular lesions somewhat comparable to those observed in intracapillary glomerulosclerosis in humans,[22] we did not find significant alteration of glomeruli in animals that received cortisone. Only one animal so treated exhibited a focal intracapillary accumulation of lipid in a renal glomerulus. A similar alteration has been observed occasionally in untreated c-f rabbits. Therefore, we hesitate to ascribe any particular significance to this isolated feature. It should also be noted that Bloodworth and Hamwi[22] observed maximum frequency of "diabetic-like" lesions in c-t rabbits after 21 to 40 days of treatment and a decline in their incidence thereafter. These lesions might not be expected in our animals since the experimental period was longer and cortisone administration was not as continuous as employed by these investigators. The failure to note significant hyperglycemia in c-t animals at the time of sacrifice is also similar to the observations of the latter investigators,

as well as Gordon and co-workers.[6] It appears worthy of note that the different effect of cortisone on glucocorticoid metabolism in the rabbit from that in the bird has been suggested as an explanation for the different effect of this hormone on cholesterol atherosclerosis in the cockerel and the rabbit. Cortisone has been noted to aggravate the atherosclerotic process in the former.[23]

The observation that hypertension overcomes the inhibitory effect of cortisone on cholesterol atherosclerosis provides information not only concerning the mechanism of cortisone action in this regard but also that related to the general problem of atherogenesis, at least as it pertains to this species. A direct relationship between the degree of atherosclerosis and aortic ground substance was observed in this study. The administration of cortisone to normotensive c-f animals receiving a dietary supplement of cholesterol resulted in less aortic acid mucopolysaccharide than was observed in untreated c-f rabbits. Concomitantly, aortic atherosclerosis was less in c-t, c-f rabbits than in untreated c-f animals. On the other hand, hypertensive c-t, c-f rabbits exhibited comparable degrees of aortic atherosclerosis and metachromasia to that observed in the latter. The greatest degrees of aortic atherosclerosis and metachromasia were observed in hypertensive, untreated c-f animals. Parallel observations of change in aortic ground substance in cortisone-treated and untreated rabbits maintained on the stock, cholesterol-free diet indicate that such changes are not dependent upon the atherosclerotic process *per se*. The decrease in aortic acid mucopolysaccharide observed in c-t animals is in keeping with the well recognized effect of this hormone on this substance in other connective tissue sites. These findings reaffirm previous observations made in this laboratory on the effect of hypertension on cholesterol atherosclerosis in the rabbit and support the consideration that an apparent increase of aortic acid mucopolysaccharide represents a morphologic expression of hypertension in this structure.[13] The association of increased aortic ground substance and an accentuation of the atherosclerotic process has been demonstrated in frozen-dried homografts,[24] lathyrism,[25] and aortic cauterization[26] in cholesterol-fed rabbits and pyridoxine deficiency in the monkey.[27] Although the precise role of the ground substance in atherosclerosis has not been elucidated, Faber[28] has suggested that this material might have a heparin-like action and thus facilitate the release of lipid from lipoprotein. It is of interest that although alloxan diabetes also inhibits cholesterol atherosclerosis in the rabbit, no effect on aortic ground substance could be discerned in such animals.[14] However, the successful induction of hypertension in such animals resulted in a more severe degree of atherosclerosis than was observed in normotensive c-f

rabbits, and aortic mucopolysaccharide was greater in the hypertensive animals. This information indicates that the mechanisms involved in the inhibition of aortic atherosclerosis by diabetes and cortisone in the rabbit are divergent. However, both may be overcome by hypertension, and the greater degrees of atherosclerosis encountered are associated with apparent increases in aortic ground substance.

Alterations observed in the serum lipids in this investigation, which might play a role in the action of cortisone on the atherosclerotic process in the rabbit, warrant consideration. The demonstration that cortisone administration to c-f rabbits resulted in hyperlipemia characterized by a relatively low degree of hypercholesteremia but a marked increase of other lipid components has been observed by others.[4–6,9] This lipid profile has prompted the suggestion that the inhibition of atherosclerosis in c-t, c-f rabbits may be directly related to the lower levels of cholesterol and depressed cholesterol:phospholipid ratios. It has also been demonstrated that cortisone administration results in a decrease of atherogenic molecules of the S_f 80 and lower classes[10] which might also contribute to the inhibitory effect noted. It appears plausible to assume that all of these considerations, in conjunction with the alterations of aortic ground substance, may be mutually significant in accounting for the effect of cortisone on cholesterol atherosclerosis. However, it appears noteworthy that despite similar plasmatic lipid relationships in hypertensive and normotensive, c-t, c-f rabbits, atherosclerosis was more severe in the former. This information indicates the greater significance of aortic composition in accounting for the inhibitory effect of cortisone on cholesterol atherosclerosis and demonstrates that atherosclerosis may be produced in animals despite a relative stability of serum cholesterol. The proposed relationship of the effect of the cortisone on aortic acid mucopolysaccharide and cholesterol atherosclerosis is not totally incompatible with the concept attributing the effect of this agent on the atherosclerotic process to reduced tissue permeability. However, if decreased aortic mucopolysaccharide signifies reduced tissue permeability, one must assume that hyaluronidase of tissue or other source is responsible for normal permeability since the anti-spreading action of cortisone apparently results from the suppresssion of hyaluronidase substrate, acid mucopolysaccharide.[29] On the other hand, absolute permeability in the absence of hyaluronidase appears accentuated following the administration of cortisone.[30] This consideration indicates the necessity for further exploration of factors concerned with the permeability of aortic tissue in normal as well as pathologic states before the inhibitory action can be unequivocally ascribed to such a mechanism.

Since alterations of body weight were similar in hypertensive and normotensive, c-t, c-f rabbits, little significance can be attributed to under-nutrition as playing a significant role in the mechanism of cortisone inhibition of cholesterol atherosclerosis.

Summary

The inhibition of cholesterol atherosclerosis in the rabbit by the administration of cortisone has been reaffirmed. However, the successful induction of renal hypertension overcomes this phenomenon. Hypertensive, cortisone-treated (c-t), cholesterol-fed (c-f) rabbits exhibited a comparable degree of aortic atherosclerosis to that noted in untreated normotensive c-f controls although less aortic alteration than was observed in untreated, hypertensive c-f rabbits. These differences appear related to changes in the histochemically demonstrable acid mucopolysaccharide content of the aorta. Cortisone effects a reduction in this material which is evident in c-f rabbits as well as in those maintained on a stock laboratory diet. On the other hand, hypertension results in an apparent increase of aortic acid mucopolysaccharide in c-t, c-f rabbits comparable to that in c-f controls.

A decrease in cholesterol:phospholipid ratios and a relatively lower level of hypercholesteremia are observed in c-t, c-f rabbits. Although these plasmatic factors may also be significant in accounting for the inhibitory effect of cortisone on the atherosclerotic process, the different degrees of atherosclerosis in c-f, hypertensive and normotensive animals suggest that the effect of this agent on aortic ground substance may be of greater significance since serum lipids were comparable in these groups. However, the mechanism by which aortic ground substance influences the atherosclerotic process remains to be elucidated.

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LEGENDS FOR FIGURES

FIG. 1. Sections of aortic arch stained with thionine. The varying degrees of metachromasia (appearing gray) are evident. × 60.

A. Cortisone-treated, cholesterol-fed.

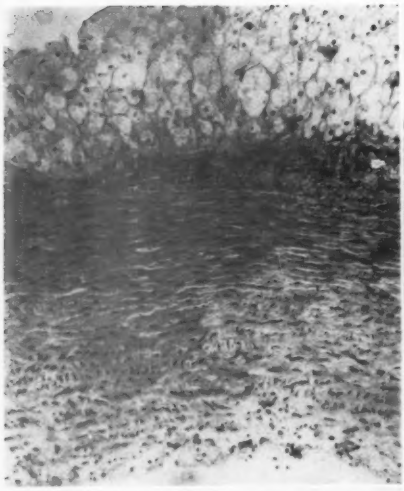
B. Untreated, normotensive, cholesterol-fed.

C. Cortisone-treated, hypertensive, cholesterol-fed.

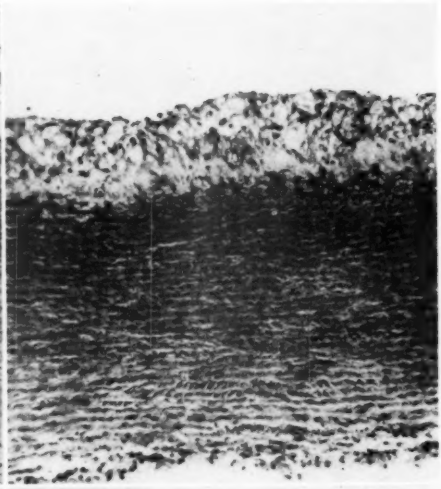
D. Untreated, hypertensive, stock diet.

1A

1C



1A



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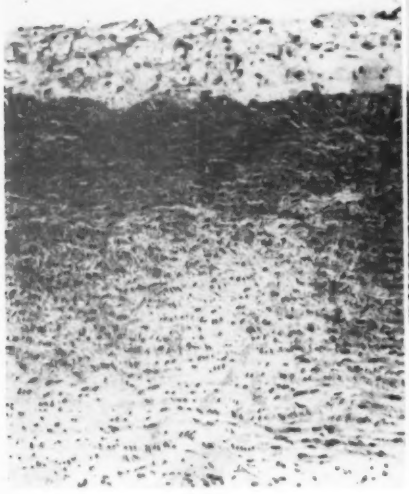

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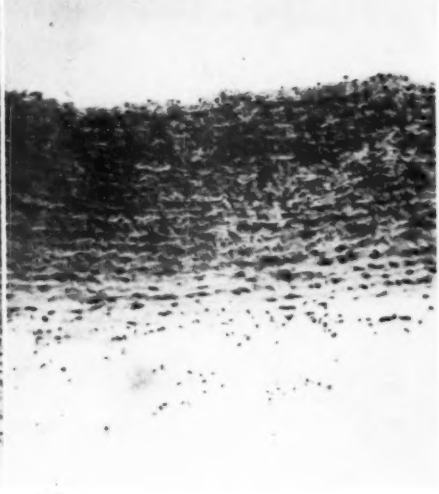

1D

NEPHROTOXIC SERUM NEPHRITIS IN THE RAT

Electron and Light Microscopic Studies

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Anti-kidney serum nephritis was first produced in rats by Masugi.[1] Subsequent investigators[2,3] pointed out that the experimental disorder caused relatively mild "nephritic" symptoms (hematuria, hypertension, azotemia), and much more prominent "nephrotic" manifestations (proteinuria, hypoproteinemia, lipemia, edema). During the nephrotic stage, the glomerular lesions were generally mild and often inconspicuous as seen in conventional histologic preparations. The disease in the rat has therefore been compared with so-called lipoid nephrosis in man.[2] The pathologic features of lipoid nephrosis and of nephrotic glomerulonephritis in man are now under intensive study by a number of investigators, including electron microscopists. Various alterations have been observed; among these are disappearance of the epithelial foot processes in the glomeruli,[4,5] thickening and mottling of the capillary basement membranes,[4,5] splitting of the basement membranes,[6,7] and deposition of "hyalin" (protein) between the basement membranes and the epithelium.[7-9] Electron microscopic studies[10-18] indicate that similar lesions may also be found in experimental nephritis. We have examined the glomerular changes in nephrotoxic serum nephritis in the rat by light and electron microscopy. The present report deals with the observations during the first 6 weeks of the experiment.

Material and Methods

Anti-rat kidney serum was prepared by immunizing rabbits according to the procedure recommended by Heymann.[19] When first collected, the serum exhibited considerable "primary" toxicity. After the usual method of partial detoxification for ½ hour at 56° C., the serum was immediately subdivided into small vials and kept in the freezing compartment of a household refrigerator for 1 to 2 months. By that time toxicity was considerably reduced. The serum potency was then tested and if satisfactory, the whole batch was transferred into a freezer at −20° C. Under these conditions the serum kept well for as long as 3 years.

Male Sprague-Dawley albino rats, weighing 100 to 150 gm., were given a single

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intravenous dose of a potent serum, 0.7 to 1.8 ml. per hundred gm. of body weight. The animals were kept in individual metabolic cages and offered food (Purina Chow) and water *ad libitum*. Urinary volume, total protein excretion, and body weight were measured daily. The normal urine volume for the strain used was 5 to 7 ml. in 24 hours; total protein excretion was up to 10 mg. in 24 hours. The latter was measured by a modification of the biuret method of Kingsley.[30] Blood and urine were also collected for paper electrophoresis, and nonprotein nitrogen (N.P.N.) was determined at intervals or at the time of sacrifice, using a modification of the standard nesslerization procedure of Folin and Wu.

Experimental nephritis was induced in over 100 animals. They were sacrificed by exsanguination or died spontaneously between 2 hours and 6 weeks after the injection. In all of these, the tissues were examined using conventional histologic procedures after fixation in 4 per cent, cold (5 to 10° C.) neutral buffered formaldehyde. In 25 of the animals, kidney tissue was also fixed immediately in cold buffered (pH 7.4) 2 per cent osmium tetroxide for electron microscopy, and thin (0.5 μ) sections were prepared for light microscopy. Sections intended for light microscopy, whether standard or thin, were stained with hematoxylin and eosin, periodic acid-Schiff (PAS) reagent, Jones's periodic acid-silver methenamine (PA-SM) and a modification of Mallory's aniline blue, chromotrope-aniline blue (CAB), as used in previous studies.[31] Osmium-fixed tissue was embedded in butyl methacrylate and sectioned with a Porter-Blum microtome. Electron microscopy was performed with the Phillips EM 100 B microscope, equipped with a 35-mm. camera. All micrographs were taken on Eastman Kodak Spectroscopic film (#6490).

Results

Clinical Data

As might be expected, the course of the experimental illness and the severity of the anatomic changes varied considerably with the dose of the anti-kidney serum. A large dose induced an almost immediate oliguria with the urinary output dropping down to 2 ml. or even less than 0.5 ml. in 24 hours. Even complete initial anuria has occurred in some instances.[12] Some of the animals died during the first 3 days, apparently in uremia. The oliguria persisted in varying degree for 4 to 5 days and was followed by diuresis, up to 25 ml. per 24 hours, and general clinical improvement. Thereafter, persistent polyuria (10 to 12 ml. per 24 hours) developed in some animals.

Oliguria went hand in hand with rapidly increasing subcutaneous edema and ascites. Weight gain became apparent in the first 24 hours of illness, reaching a maximum during the first week. The largest gain was 100 gm., nearly equal to the initial body weight; the average gain was 50 to 60 gm., 40 to 50 per cent of body weight. This was caused almost entirely by accumulation of water, because the animals ate very little but continued to drink. If diuresis occurred, edema disappeared in the course of 2 to 4 days. If diuresis did not occur, edema increased and the animal died apparently in cardiac failure, but with only moderate nitrogen retention (N.P.N., 75 to 85 mg. per hundred cc.).

Proteinuria appeared almost immediately after injection of the serum. Specimens collected for the first 3 hours contained several gm. per hun-

dred ml. Protein loss was accompanied by a rapidly developing hypoproteinemia with serum levels dropping below 2 gm. per hundred ml. by the third or fourth day. During the ensuing week serum proteins showed partial recovery despite continued or even increasing proteinuria. This gradually declined after the second week, but most animals alive at 6 weeks still showed appreciable loss of protein and some degree of hypoproteinemia.

Nitrogen retention was apparent 6 hours after injection of the serum, increasing rapidly up to 100 to 200 mg. of N.P.N. by the end of the third day. If the rat did not die at that time, the level of N.P.N. began to decrease, reaching normal or even subnormal levels in some animals in 2 to 4 weeks. This drop occurred even in the rats that did not develop diuresis. However, in the animals that showed persistent proteinuria, elevated N.P.N. was seen even at 6 weeks.

Microscopic hematuria and casts appeared during the first week in most of the animals. None showed gross hematuria.

Anatomic Lesions

The histologic description is based upon the maximal damage observed and represents a composite of the standard sections, thin sections and electron micrographs. The standard sections, particularly those stained with PAS, provided an over-all view of the renal alterations and their distribution in the glomeruli, the cortical tubules and the medulla. The thin sections were cut from the same blocks utilized for electron microscopy; this again allowed us to judge the uniformity of lesions within the glomeruli and to correlate the light microscopic appearance with the electron microscopic pattern.

Anatomic alterations in the glomeruli appeared very soon after the injection of serum. Piel[17] observed them within the first hour. In our experiments, noticeable swelling of the cytoplasm of the epithelial and the endothelial cells, particularly of the attenuated layer (*membrana attenuata*) and partial disappearance of the endothelial pores was present 2 hours after the injection. The capillary lumens contained many neutrophils and also mononuclear blood cells, the former often adherent to the capillary walls (Fig. 5). The tubules showed no significant changes at this time. At 6 hours, cellular edema became much more pronounced. The epithelial foot processes were swollen and distorted and in places completely absent. There was also swelling of the endothelial cells lining the capillary plexus at the junction of the cortex and the medulla.[22] The proximal convoluted tubules contained a small number of hyaline droplets. In one animal, the inner zone of the medulla and the papilla were almost completely filled with pale hyaline casts.

At 24 hours the glomeruli appeared quite bloodless with but a few red

cells, neutrophils and mononuclear leukocytes in the capillaries. The lumens of the latter were almost completely obliterated by tremendously swollen vacuolated endothelial cells (Fig. 10). The urinary space was markedly narrowed by swollen epithelium (compare Figs. 1 and 2). The foot processes had almost completely disappeared, and the uniform, pale cytoplasm was directly applied to the basement membrane (Fig. 10). Even the mitochondria appeared swollen. Occasional red cells were seen in the urinary space between the epithelial cells. The capillary basement membranes appeared altered; the dense middle layer was irregularly thickened, and the paler layers were uneven (compare Figs. 9 and 10). Thickening of the basement membrane was observed by Piel[12] as early as 6 hours after the introduction of serum. Some capillaries contained "hyaline" thrombi which, under the electron microscope, had a fibrillar structure (Figs. 11 and 12). These fibrils exhibited a dominant periodicity of 230 A; there was also a suggestion of two shorter periods, at about 160 Å and the other less than 100 A. The endothelium and, to a lesser degree, the epithelium of the thrombosed capillaries were destroyed. The proximal convoluted tubules contained numerous hyaline droplets. A number of casts which varied in their affinity for PAS stain were seen in Henle's loops and convoluted tubules. Protein precipitate appeared in the corticomedullary capillary plexus.

By the third day the cellular changes were at a maximum. In the most severely affected animals, many glomeruli were filled with "hyaline" thrombi which almost completely replaced the endothelial cells (Fig. 2). Where the latter were preserved, they contained many vacuoles, while osmiophilic droplets lay within the cells or between them and the basement membranes. The epithelium was devoid of foot processes, but often showed long thin interlacing projections crossing the urinary space (Fig. 13). In addition to vacuoles the cells contained hyaline droplets and fat droplets. The basement membranes were distinctly thickened, with focal splitting of the dense layer. There were fewer hyaline droplets but more casts in the convoluted tubules. The peritubular capillaries contained a number of free-lying mononuclear cells, and there was an increased number of cells in the interstitial connective tissue.

In the animals which improved clinically, the glomerular cells began to recover after the third day. At 6 days the cytoplasmic swelling decreased and the epithelial foot processes re-appeared though they were distorted and focally fused. The capillary lumens became patent. Thickening and splitting of the basement membrane increased, and for the first time there was widening of the intercapillary spaces (Fig. 3) with increase in cells and basement membrane branches (Fig. 6). If, on the other hand, there was no diuresis and no reduction in edema, the glo-

merular changes continued unabated. In animals sacrificed in a terminal state on the eighth day, the foot processes were either markedly distorted or nearly totally absent. Deposition of a dark substance with the size and shape of the foot processes within the epithelial cytoplasm near the basement membrane was noted (Fig. 14). It could have represented "attempts" to reform the foot processes. This substance ("foot process material") was considerably denser than the basement membrane but was similar to the substance normally present in the foot processes. A few fibrin thrombi still filled the capillary lumens. Splitting of the basement membrane reached the stage where it could be recognized in thin sections by means of the light microscope (Fig. 7).

Animals sacrificed during the second to the fourth week showed only slight to moderate cellular edema and distortion of foot processes. There was progressive thickening of the basement membrane up to 5,000 Å (normal width, 800 to 1,200 A) and extensive splitting. In addition to cells and basement membrane branches (BMB), bundles of very fine fibrils appeared in the widened intercapillary spaces. Small crescents composed of the epithelium of Bowman's capsule were present in some instances.

At 6 weeks, at the termination of the experiment, the cell and foot process changes persisted to a degree (Figs. 4 and 15) while the basement membrane alterations showed further progression. The two split layers of the dense zone were now as much as 5,000 A apart at some points (Fig. 16), though often linked by connecting strands (Fig. 15). Three or 4 layers were seen occasionally. In addition, the outer split layer was often broken up into irregular fragments which lay perpendicularly to the capillary wall, while the epithelial cytoplasm in the form of thick trabeculas extended between these fragments to the inner split layer (Figs. 8 and 17).

Comment

There are only a few published reports of electron microscopic observations in anti-kidney serum nephritis; those of Simer [10,11] and Reid [13] in mice, those of Sakaguchi, Suzuki and Yamaguchi,[14] and Churg, Mautner and Grishman [16] in rabbits, and those of Piel and associates,[12,17] Miller and Bohle,[15] and Churg, Grishman and Mautner [18] in rats. These studies supplement each other and reveal basic similarity of the glomerular lesions in the several species. Cellular damage, inflammation, basement membrane changes and alterations in the intercapillary space are invariably present, differing only in degree and in the rate of development. In the rat, inflammation is mild and intercapillary

changes develop slowly while cellular and basement membrane lesions are prominent from the start.

Cellular Alterations

Swelling and vacuolation of the endothelium appeared in the rat almost immediately after the injection of serum, reaching a maximum between 1 and 3 days and declining thereafter. In severely affected animals these features were present in some degree at the end of 6 weeks. Osmiophilic lipid droplets were seen in the cytoplasm after the third or fourth day, perhaps a reflection of both the cell damage and the elevation of lipids in the blood.

Endothelial lesions have been observed in mice[10,11,13] and rats.[12,15] They have long been recognized by conventional microscopy[1-3] though their severity was not appreciated. At the height of damage, swollen endothelium almost completely fills the capillary lumen. This may well be responsible for the early oliguria and azotemia, though interference with passage of filtrate across the altered capillary wall could contribute significantly, or even predominantly. The initial vascular spasm[23] and the thrombi in the capillary lumens may also play a role. The appearance of thrombi in the capillaries has been related to the destruction of the endothelium.[1] The thrombi give a positive staining reaction for fibrin[1,24,25] and have a finely fibrillar structure with the typical periodicity of fibrin (230 A). Their similarity to the glomerular thrombi encountered in the generalized Shwartzman phenomenon has been commented upon by many observers.[12,26] Recent electron microscopic studies showed that the fibrillar substance observed in the Shwartzman phenomenon has a periodicity only about half as long (120 Å) as that of fibrin.[27]

Swelling and vacuolation of the visceral epithelium occurred simultaneously with similar alterations in the endothelium. Changes in the foot processes were first seen by Simer[10,11] and were confirmed many times in both experimental[17] and human[4,5] tissues. Swelling, distortion and disappearance of the foot processes in the rat were most evident 1 to 3 days after the injection of serum. The dark substance within the processes (foot process material) disappeared at the same time (Figs. 10 and 11). Simer[11] also noted a decrease in electron density of the pedicels in nephritic mice. Restoration of foot processes was accompanied by reaccumulation of the dark substance; however, in some of the more severely damaged animals, the processes might not be completely restored; yet the dark substance accumulated within the cytoplasm close to the cell membrane, in the form of bands and masses. It is possible that

this substance has functional significance.[4] In severe but nonfatal disease some foot process change was still seen at 6 weeks.

An interesting finding was the formation of long slender cytoplasmic protrusions which coursed the urinary space and formed an intricate network. They were first seen about the third day of illness when the foot process changes and cellular edema were at the maximum. Similar protrusions were observed by Sakaguchi and co-workers in nephritic rabbits.[14] Hyaline droplets appeared in the glomerular epithelium on about the third day; at the same time they began to disappear from the tubules.

Basement Membrane

Thickening and splitting of the basement membrane in experimental as well as in human nephritis has been recognized by both light [6,7,25,28,29] and electron microscopists.[5,10–12,14] Sakaguchi and co-workers [14] stated that in rabbits, thickening was accompanied by a decrease in electron density of the membrane. Splitting of the dense middle layer was a more advanced lesion. It has been observed in rats and rabbits [16–18] and in man in the course of the nephrotic syndrome.[30] Subdivision of the dark zone into two layers was seen faintly in electron micrographs of normal glomeruli and could be very well demonstrated by maceration and silver impregnation in a mild alkaline solution (Gomori's silver methenamine).[31] The process of splitting suggests laminated construction of the glomerular basement membrane, similar to that of the tubular basement membrane.[5,7] It has been postulated that the basement membrane of glomerular capillaries consisted of two fused elements, endothelial and epithelial.[32–36] The generic relationship of these elements to the corresponding cells is not known, nor is it yet clear whether the splitting seen by light microscopy (Fig. 7) and that observed by electron microscopy (Fig. 15) represent the same phenomenon.

Fragmentation of the outer split layer of the basement membrane was a late feature. The tendency of the fragments to arrange themselves perpendicularly to the surface of the capillary, between protrusions of the epithelial cytoplasm, produced an appearance that was not unlike that seen in so-called "membranous transformation" in the nephrotic syndrome of man.[8,37] The mechanism of this phenomenon and its possible significance in human nephrosis are currently under investigation.

Intercapillary Space

The existence of the intercapillary space has recently received support from several electron microscopic studies of the normal glomerulus.[36,38,39] The pathologic lesions encountered by us first became

apparent toward the end of the initial week, and progressed slowly for the next 5 weeks. Cells and branches of the basement membrane became somewhat more numerous, and bundles of fine fibrils were seen between the cells. The basement membrane branches corresponded to the "basement membrane-like material" of Farquhar, Vernier and Good.[4,5] Electron microscopy of silver-impregnated tissue showed that they originated from the basement membranes and ran through the intercapillary spaces in the manner of tortuous cross-beams.[31] They separated, though incompletely, the intercapillary cells from each other and from the endothelial cells. The fibrils were very delicate; they sometimes revealed a suggestion of periodic structure, but further data are needed to establish their nature and their relation to the basement membrane branches. These will be gathered as part of the study, now in progress, of the chronic stage of experimental nephritis.

Relation of Anatomic Alterations to Edema and Proteinuria

Farquhar and co-workers[4,5] called attention to the association of foot process changes with the nephrotic syndrome in man. In the rat, edema appeared almost simultaneously with the alterations of the foot processes and began to clear when foot process recovery became evident. Failure of recovery was associated with further retention of water. Though the edema was undoubtedly caused by hypoproteinemia, general capillary damage and also injury of the glomerular capillary walls could be the contributing factors. It has been claimed that the foot processes assist in glomerular filtration,[40] and it is possible that their injury, manifested by the disappearance of the dark foot process substance, interferes with transfer of water and sodium across the capillary basement membrane. The onset of edema within the first 24 hours of illness and its eventual disappearance despite continued protein loss, was reminiscent of the "nephritic" rather than the "nephrotic" syndrome in man,[41] particularly since it was accompanied by reduced rather than increased or normal glomerular filtration.

It has not been definitely established whether the glomerular ultrafilter which separates water and crystalloids from the plasma proteins is represented by the cytoplasm of the endothelial and particularly the visceral epithelial cells or by the dense layer of the basement membrane,[4,5] though the weight of evidence appears to be in favor of the latter.[42] Proteinuria of the nephrotic syndrome in man is accompanied by changes in both structures,[4,5] by fusion of the foot processes and by thickening and mottling of the basement membrane. Spiro[42] has described actual defects in the basement membrane, which he has held to be responsible for the proteinuria. In the nephritic rat, the initial pro-

teinuria was also associated with loss of foot processes and with changes in the basement membrane. In the later stages, loss of protein persisted despite recovery of the foot processes and eventually abated despite progression of the basement membrane damage. It is possible that a certain combination of changes in both the epithelial cells and the basement membrane is necessary to cause proteinuria, or that the increased porosity of the capillary wall is the result of molecular rearrangement at a sub-electron-microscopic level.

Summary

Masugi-type nephritis was induced in rats by a single intravenous injection of anti-kidney serum derived from rabbits. The clinical behavior of the animals and the anatomic lesions in the kidneys, particularly in the glomeruli were followed for a period of 6 weeks.

The animals developed mild hematuria, transient nitrogen retention and pronounced proteinuria and edema. The glomeruli showed cellular damage with acute inflammation and fibrin thrombi. There were also thickening and splitting of the capillary basement membrane with fragmentation of the outer split layer, widening of the intercapillary spaces with mild cellular proliferation, increase in basement membrane branches and deposition of fine fibrils. Relation of the anatomic to the clinical features has been discussed briefly.

Review of the pertinent literature on anti-kidney serum nephritis indicates basic similarity of glomerular lesions in the animals of several species.

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The technical assistance of Messrs. M. Gioia and A. Prado and Miss Ruby Tamura is acknowledged with pleasure.

[*Illustrations follow*]

Legends for Figures

All figures represent part or all of the rat glomerulus. Time given is that elapsed after the intravenous injection of anti-kidney serum.

Figures 1 to 4: Light photomicrographs, standard sections, periodic acid-Schiff (PAS) stain. × 450.

Fig. 1. Normal glomerulus.

Fig. 2. Three days. The bottom glomerulus shows swelling of cells and loss of capillary lumens; the top glomerulus exhibits obstruction of the lumens by "hyaline" thrombi and disappearance of most of the cells.

Fig. 3. Two weeks. There is focal thickening of the capillary walls and widening of the intercapillary spaces. A small epithelial crescent is seen on the right.

Fig. 4. Six weeks. Capillary lumens are for the most part patent; their walls are thickened. There is a capsular adhesion near the top of the glomerulus.

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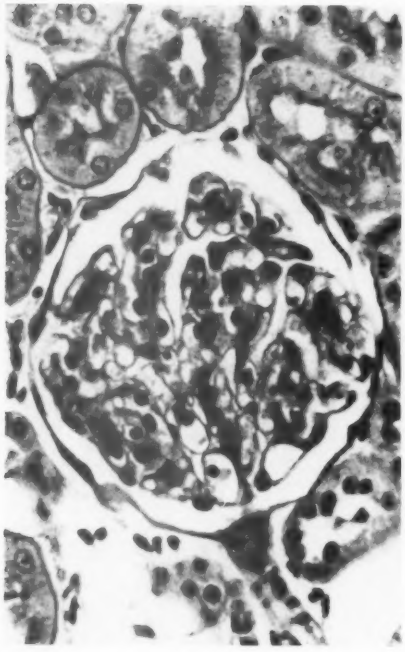

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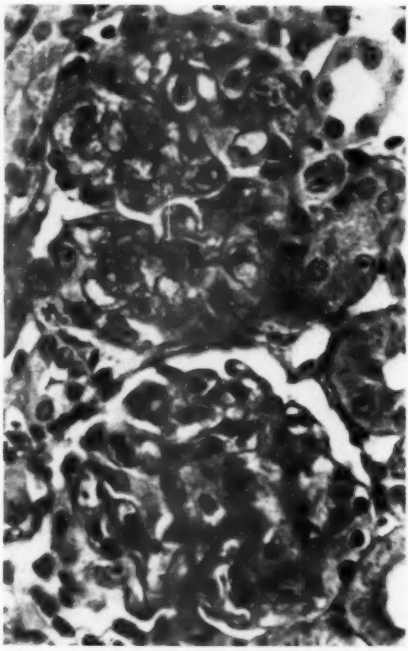

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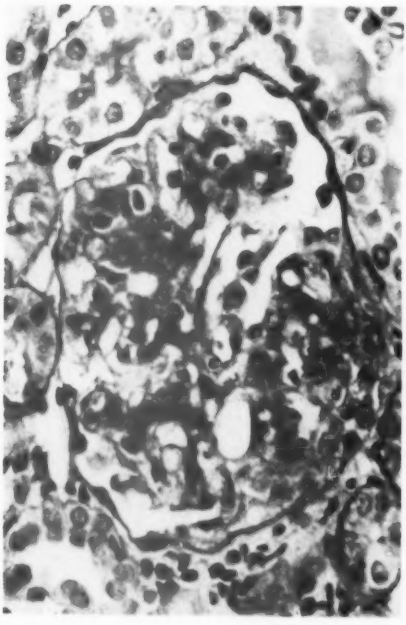

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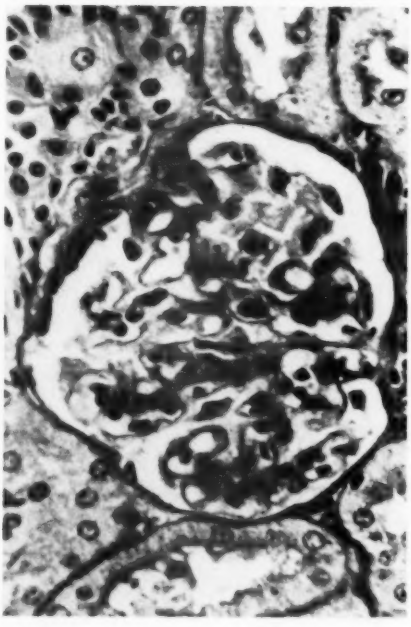

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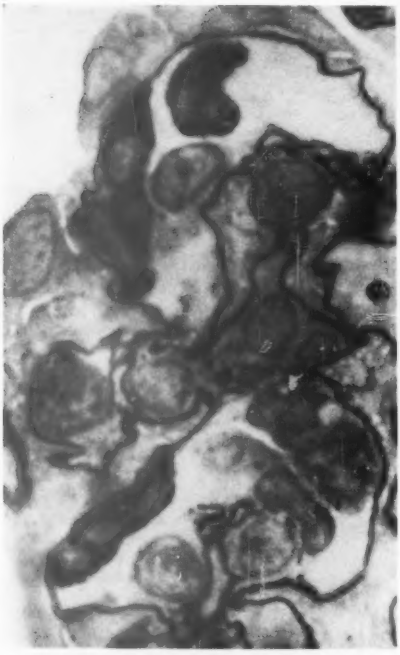
Figures 5 to 8: Light photomicrographs, thin (0.5 μ) sections, PAS stain.

FIG. 5. Two hours. Many neutrophils and mononuclear leukocytes are evident in the capillary lumens. The darkly granulated neutrophils adhere to the capillary wall. × 2200.

FIG. 6. Six days. Swelling of epithelial and endothelial cells is manifest, and there is an increased number of intercapillary cells. × 1600.

FIG. 7. Eight days. Splitting of the capillary basement membranes is shown. × 1600.

FIG. 8. Six weeks. There is splitting and fragmentation of the basement membrane of the lower capillary with formation of hair-like projections (arrow). × 1600



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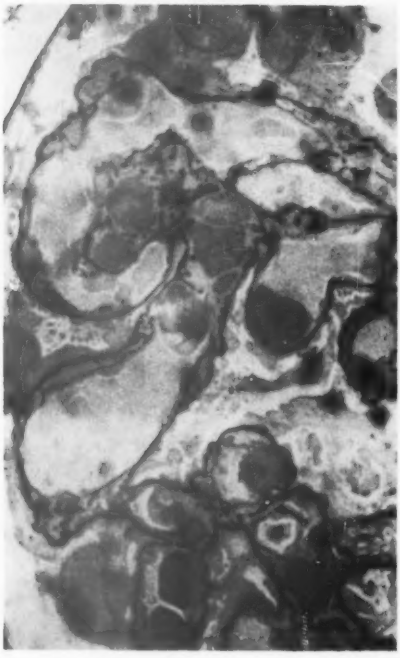

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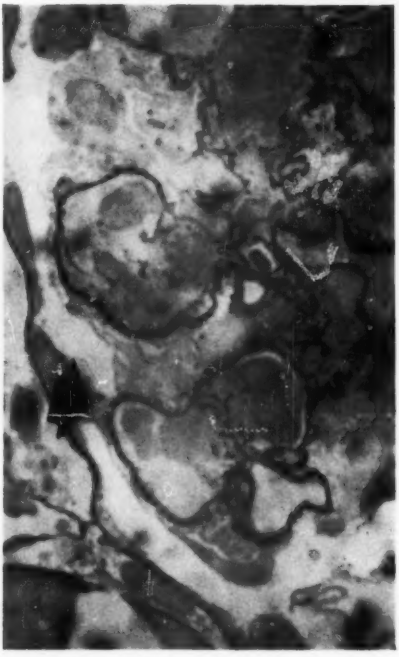

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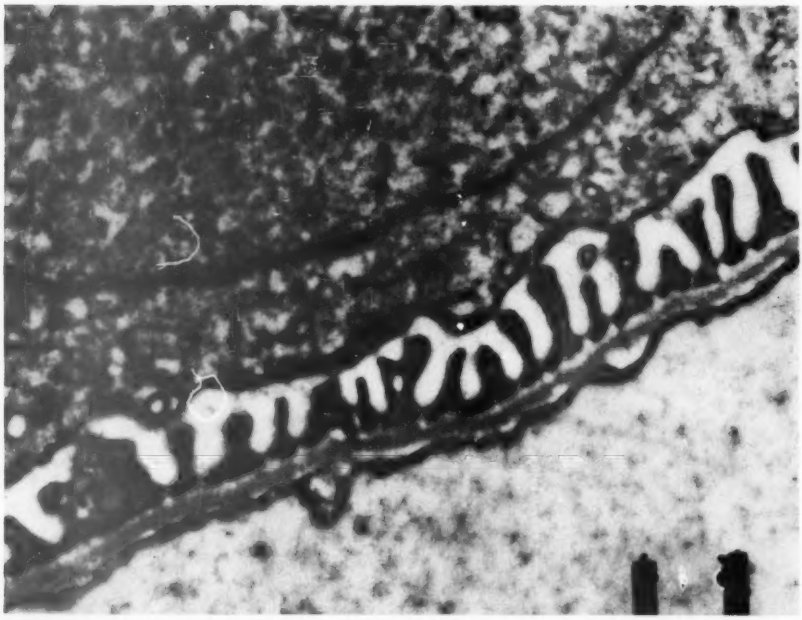
Figures 9 to 17: Electron micrographs.

FIG. 9. Capillary wall of a normal glomerulus. Shown are (from top to bottom): part of the nucleus and cytoplasm of an epithelial cell, foot processes, the 3 layers of the basement membrane and the attenuated layer of endothelial cytoplasm. × 12,500.

FIG. 10. Twenty-four hours. Swelling of the epithelial cytoplasm and complete loss of foot processes is evident in the upper right. There is swelling of endothelial cells with obstruction of the lumen (lower left) and irregular widening of the dense middle layer of the basement membrane. U, urinary space; B, basement membrane; C, capillary lumen. × 12,500.



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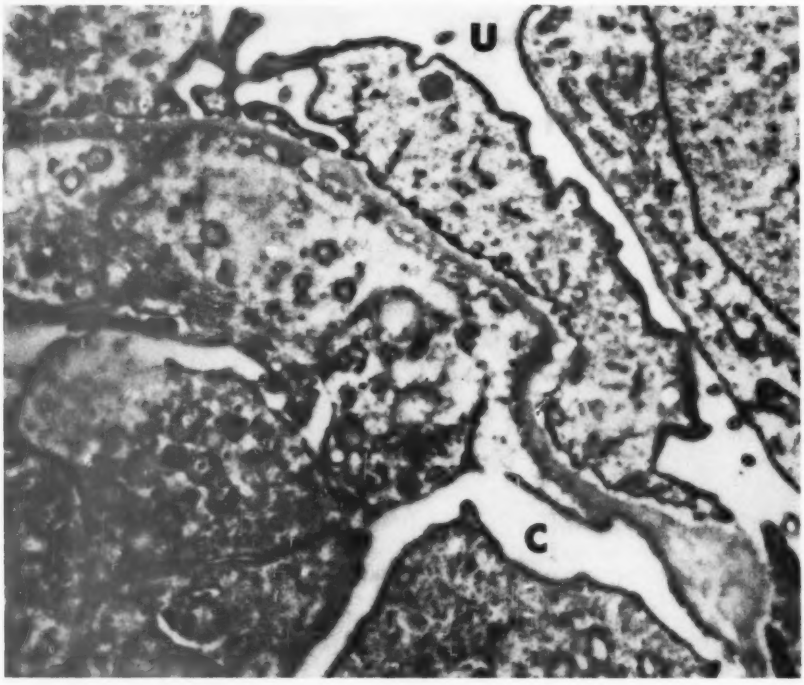

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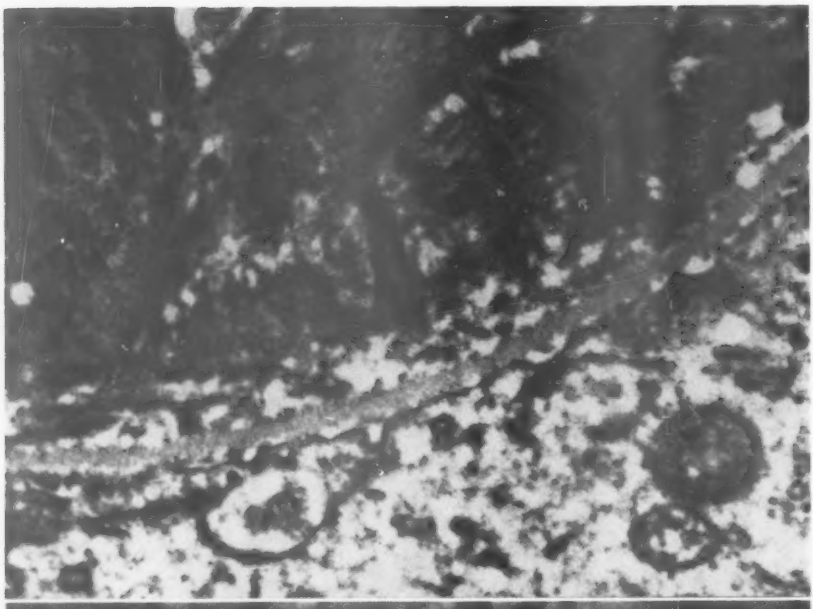

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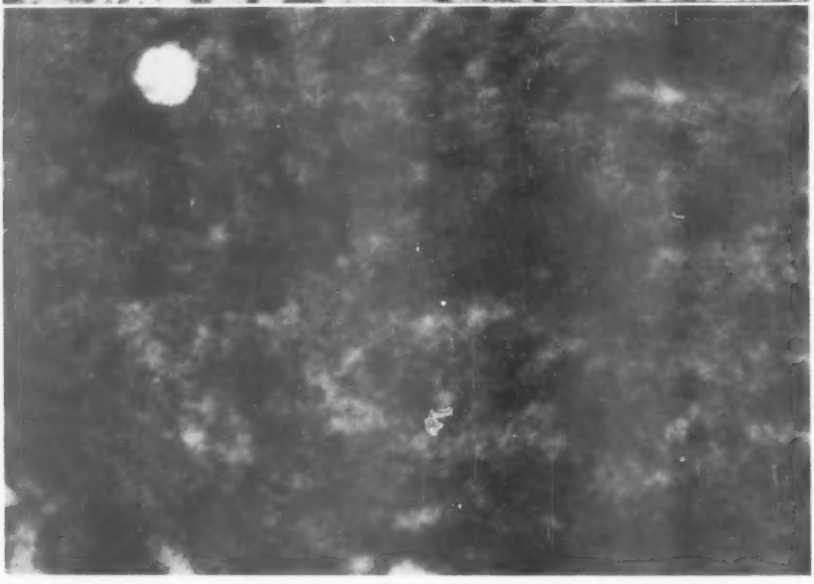

FIG. 11. Twenty-four hours. The capillary lumen is filled with a fibrillar substance, presumably fibrin. The endothelial cytoplasm is destroyed. × 25,000.

FIG. 12. Higher magnification of the previous figure, showing periodic structure of the fibrils (period equals 230 Å). × 62,500.

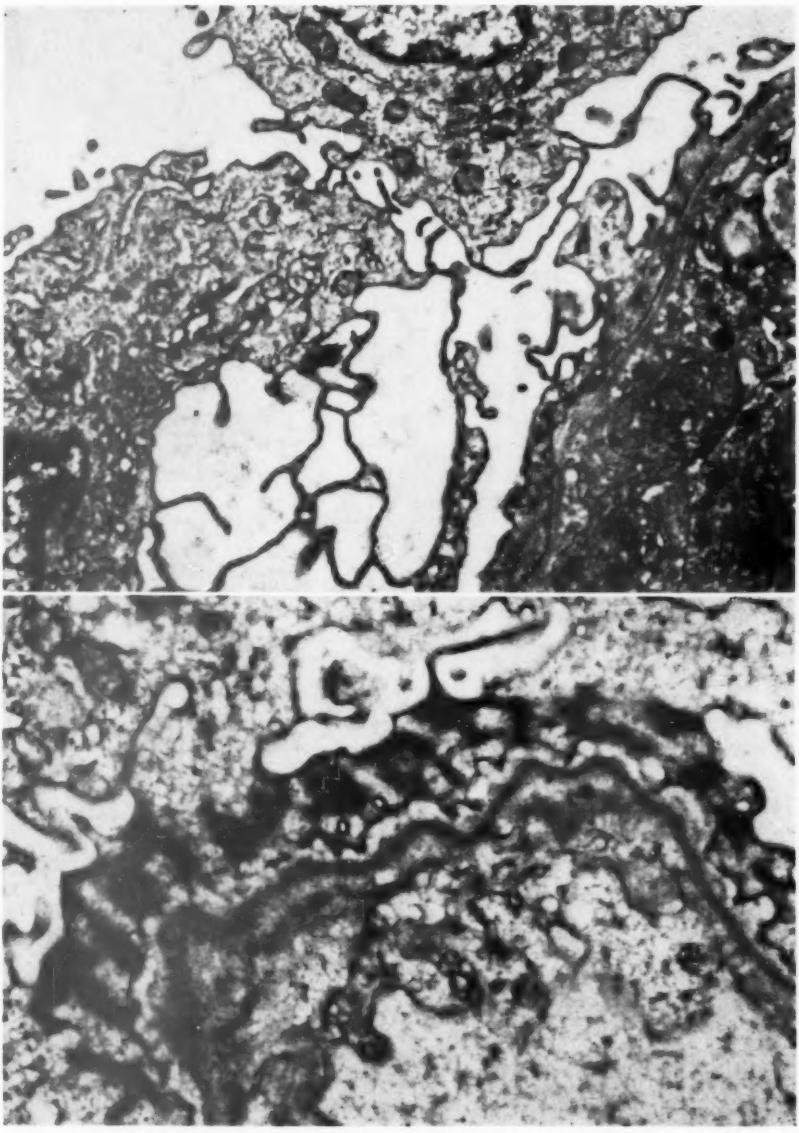

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FIG. 13. Three days. Slender interlacing projections of the visceral epithelial cells are evident. The capillary lumen in the right lower corner is filled with cells. Foot processes are absent. × 8500.

FIG. 14. Eight days. The cytoplasmic membrane of an epithelial cell is directly applied to the capillary basement membrane. Within the cytoplasm, close to the membrane, are deposits of dark substance (foot process material). × 12,500.

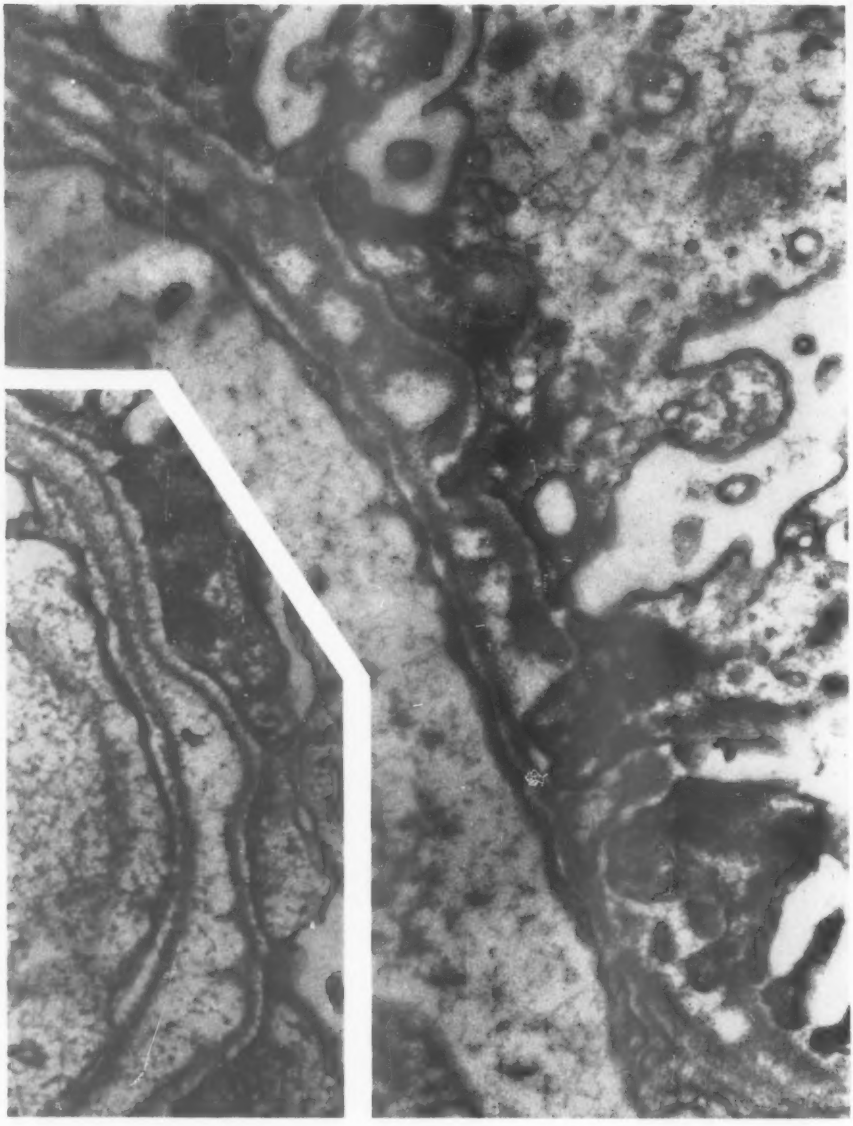

FIG. 15. Six weeks. Capillary basement membrane shows longitudinal splitting of the dense middle layer (top) and beginning fragmentation of the outer of the two split layers (bottom). × 30,000.

FIG. 16. Six weeks. Part of a capillary basement membrane exhibits wide separation of the split layers. × 30,000.

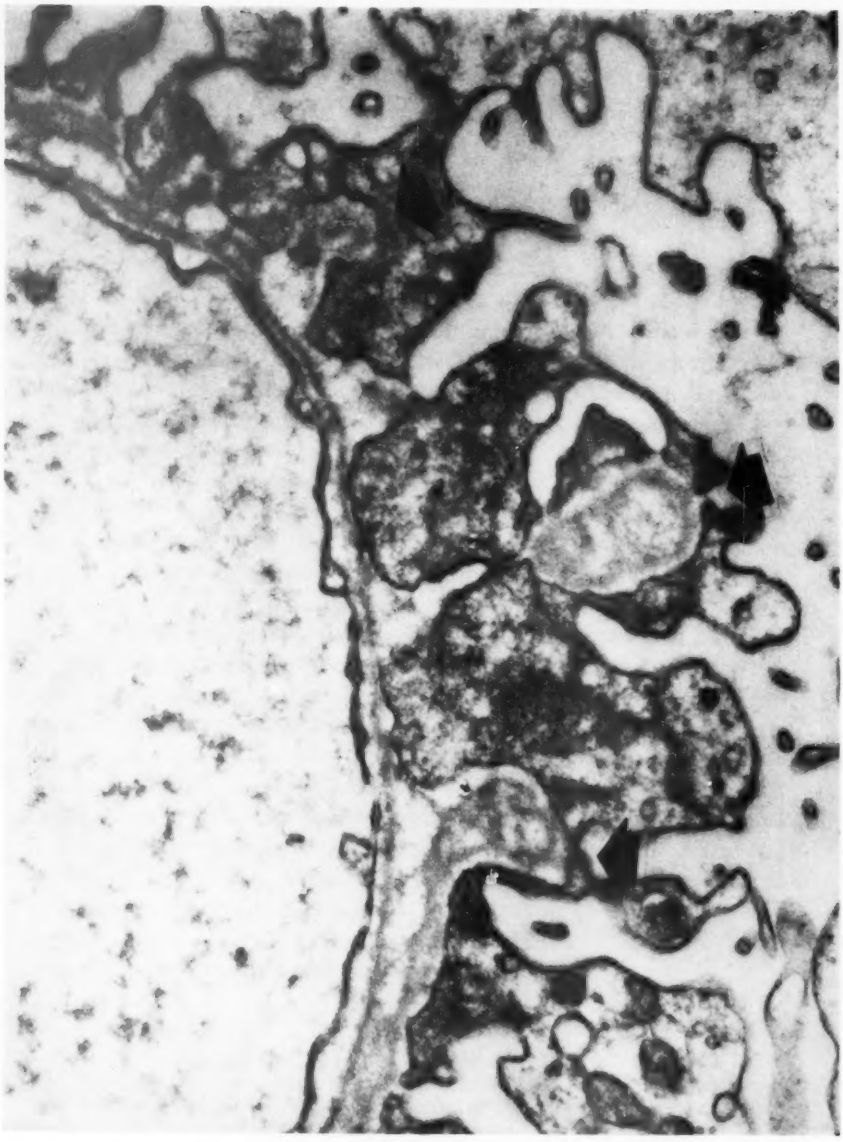

FIG. 17. Six weeks. Large epithelial trabeculas—abnormal foot processes (?)—lie between fragments of basement membrane (arrows). Two normal-sized foot processes are seen in the top left corner. × 30,000.

PRODUCTION OF OCCLUSIVE VASCULAR LESIONS BY BACTERIAL ENDOTOXIN IN KIDNEYS OF RATS WITH DEOXYCORTICOSTERONE HYPERTENSION

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Deoxycorticosterone acetate (DOCA) produces a characteristic syndrome in partially nephrectomized rats kept on a high sodium diet, which in many ways resembles the morphologic and functional aspects of human hypertensive vascular disease.[1,2] The administration of renin-containing kidney extract to such rats, according to Masson, Corcoran and Page,[3] results in an eclampsia-like syndrome with renal vascular lesions primarily involving the glomeruli. In the course of our study of DOCA hypertension in the rat, an attempt was made by various means to alter the course of the disease. This report describes the morphologic alterations noted after the administration of 5-hydroxytryptamine (5HT), bacterial endotoxins, and renin-containing kidney extracts (renin) to DOCA-hypertensive rats.

The effects of renin previously observed [3] are confirmed in this report. 5HT was given in view of its reported nephrotoxicity [4] and its suggested role in the etiology of essential hypertension [5] and eclampsia.[6] Bacterial endotoxins are known to be vasculotoxic. Their administration in various species results in renal cortical necrosis and also intravascular deposition of fibrinoid,[7] features which are at times encountered in different forms of hypertensive vascular disease. Our observations show some histologic similarities between the renal lesions caused by renin and endotoxin. Moreover, demonstrating that the renal vasculature in DOCA-hypertensive rats is unusually reactive to bacterial endotoxin, the present study may have implications in relation to the pathogenesis of human eclampsia and malignant nephrosclerosis.

Material and Methods

Carworth Farm Nelson rats of both sexes, weighing approximately 150 gm., were used. Hypertension was produced by unilateral nephrectomy, subcutaneous implantation of two 25-mg. pellets of DOCA, and the substitution of 1 per cent sodium

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chloride for drinking water. Within 3 weeks all of the animals were hypertensive, with systolic blood pressures in excess of 150 mm. of Hg. Systolic blood pressures were measured at regular intervals by the microphonic method of Friedman and Freed,[8] with the animals under light ether anesthesia.

The renal extract, containing 0.55 dog units of renin per mg., was prepared from hog kidneys by Dr. Oscar Helmer (Lilly Research Laboratories) and purified *Escherichia coli* endotoxin was prepared by the method of Webster, Sagin, Landy and Johnson.[9] 5-Hydroxytryptamine was used as the creatinine sulfate.

The schedule of treatment in the different categories is summarized in Table I.

In the first series of experiments, animals with DOCA hypertension of short duration (30 days after implantation of DOCA pellets) were divided into 4 groups. Group I consisted of untreated controls. Rats in group II received 3 subcutaneous injections of renin, 0.25 ml. (15 dog units) each, 12 hours apart. The animals in group III were given 5HT twice subcutaneously in doses of 1 mg. and 2 mg. respectively, 12 hours apart. Those in group IV received injections of endotoxin intraperitoneally in 2 doses of 1 mg. and 2 mg., respectively, 12 hours apart. Group V consisted of normotensive controls. Members of this group received renin, or 5HT, or endotoxin, according to the above-mentioned dose schedule. All surviving animals were sacrificed with chloroform 26 to 28 hours after the first injections, and necropsy examinations were performed immediately. Blocks of kidney, heart, and liver were fixed in Zenker's solution, and sections were stained with hematoxylin and eosin, and by the periodic acid-Schiff (PAS) method.

In another series of experiments, the effects of "endotoxin tolerance" and the long-term administration of endotoxin to normotensive rats with uninephrectomy and to those with DOCA hypertension were investigated. All animals listed in groups VI to IX received 400 μg. of endotoxin, intraperitoneally, weekly for 6 months. These groups consisted of normal animals divided as follows: Group VI served as controls. In group VII, rats were given 1 per cent NaCl instead of water. In group VIII they were kept on an "endotoxin tolerance regime" for 5 days (injection of 50, 100, 150, 200, 250 μg. of endotoxin daily), a procedure which makes rats resistant to normally toxic doses of endotoxin. At the end of this time, DOCA pellets were implanted subcutaneously. In group IX, in addition to the endotoxin tolerance course, rats were given 1 per cent NaCl as a substitute for water.

Groups X, XI and XII consisted of older DOCA-hypertensive animals. Group X served as controls. Group XI rats were given weekly injections of 400 μg. of endotoxin, and in group XII, in addition to the weekly injections of endotoxin, an endotoxin tolerance course was maintained before the implantation of DOCA pellets. All surviving animals in groups VI to XII were sacrificed with chloroform 6 months after the start of the experiments. Kidney, heart, liver and spleen were fixed in Zenker's solution, and sections were stained with hematoxylin and eosin and the PAS and azan-carmine stains.

Results

Short-term Experiments with Renin, 5HT and Endotoxin

Group I. The salient pathologic features of early DOCA hypertension, as described by various workers,[2,10] were reproduced (Figs. 1 and 2). Hematoxylin and eosin and PAS stained sections of kidneys revealed minimal thickening of small arteries and arterioles. In each midsagittal section of the entire kidney, one or two arterioles showed subintimal degeneration, which was PAS-positive; however, the lumens of such vessels were not compromised. The glomeruli exhibited little or no thicken-

TABLE I

EXPERIMENTAL PLAN

	No. and sex	Av. blood pressure on last day of experiment (mm. Hg)*	Av. wt. on last day of experiment (gm.)*	Duration of DOCA hypertension	Occurrence of occlusive glomerular lesion
Group I DOCA control	2 M 2 F	170	243	30 days	None
Group II DOCA + renin	3 M 3 F	172	254	30 days	4 of 6 †
Group III DOCA + 5HT	3 M 3 F	176	241	30 days	None
Group IV DOCA + endotoxin	3 M 3 F	177	235	30 days	4 of 6 ‡
Group V (normotensive) Renin or 5HT or endotoxin	2 F 2 F 2 F	115	262		None
Group VI Endotoxin control	4 F	110	290		None
Group VII Endotoxin + NaCl	4 F	116	283		None
Group VIII Endotoxin + endotoxin tolerance	4 F	116	289		None
Group IX Endotoxin + NaCl + endotoxin tolerance	4 F	122	279		None
Group X DOCA control	4 F	180	271	6 mo.	None
Group XI DOCA + endotoxin	10 F	199 §	267	6 mo.	8 of 8
Group XII DOCA + endotoxin + endotoxin tolerance	10 F	183	250	6 mo.	6 of 10

* In groups I to V data obtained refer to measurements made prior to injections.
† One animal died after first injection of renin.
‡ Two animals died after first injection of endotoxin.
§ Average in 8 animals; 2 died after 2 months of endotoxin treatment.

ing of the basement membrane. Occasional glomeruli were hyalinized, and a few showed focal necrosis of portions of their tufts. Scattered convoluted tubules were dilated and contained hyaline or red cell casts. The latter were most likely due to the focal necrosis in glomerular tufts. There was no evidence of inflammation.

Two of the 4 rats in this group exhibited small focal areas of necrosis

of myocardial fibers in the left ventricle. No lesions were noted in the livers.

Group II. The physiologic manifestations of the DOCA-renin syndrome were similar to those observed by Masson, Corcoran and Page.[11] Blood pressures measured one hour after the first injection of renin indicated an average rise of 30 mm. of Hg. The second injection induced convulsions, hematuria and anasarca in surviving animals.

Sections of kidneys (Fig. 5) revealed lesions characterized by fibrinoid degeneration of the capillary walls and basement membranes in the tufts of the majority of glomeruli in 4 of 6 rats. There was cytoplasmic degeneration of endothelial and epithelial cells especially in those portions bordering the basement membrane. This degeneration, evidenced by PAS-positive droplets in the cytoplasm, occasionally resulted in coalescence, leading to obliteration of capillary lumens. In a few of the glomeruli there were focal intraluminal deposits of homogeneous PAS-positive material with preservation of the related capillary wall. All 6 animals in this group had pathologic changes in arteries, arterioles and tubules, identical to those seen in the DOCA controls (group I).

Small foci of myocardial necrosis were seen in 2 of the 4 rats with glomerular occlusion and in 1 of the 2 rats that failed to develop this lesion. In the liver, no lesions were seen.

Group III. The only pathologic alterations in DOCA-hypertensive animals given 5HT were those seen in DOCA controls (group I). Blood pressures taken one hour after the first injection of 5HT showed an average reduction of 20 mm. of Hg.

Group IV. Endotoxin administration resulted in diarrhea, prostration, and evidence of shock in all animals within this group. An average of 50 mm. of Hg drop in blood pressure was noted one hour after the first injection of endotoxin. Such a dose (1 to 2 mg.) is rarely fatal in normotensive controls and causes only mild malaise but no evidence of circulatory collapse.

Four of the 6 animals given endotoxin revealed intraluminal deposition of homogeneous PAS-positive material in most of the glomerular capillaries. The occluding deposit was dense and resembled fibrinoid (Fig. 6). In addition, the glomerular basement membranes in these 4 rats were moderately thickened by granular masses of PAS-positive substance, similar to, but less extensive than that observed following the administration of renin (group II). Although 2 of the animals in this group failed to exhibit these lesions, they showed arterial, arteriolar and tubular alterations identical to those in DOCA controls (group I). It is interesting to note that the occlusive glomerular lesion was most marked in animals with the highest blood pressures (Table II).

In 1 of the 4 rats with the occlusive glomerular lesion and in 1 without this lesion there were small focal areas of myocardial necrosis. The liver in 1 animal showed numerous areas of centrilobular necrosis with an early neutrophil infiltration. This animal lacked the occlusive glomerular lesion but had focal myocardial necrosis. The liver damage was probably secondary to shock induced by endotoxin.

Group V. Microscopic examination of the kidney, liver and heart in normal animals receiving renin, or 5HT, or endotoxin revealed no lesions.

TABLE II

INCIDENCE OF OCCLUSIVE RENAL LESIONS IN RATS TREATED WITH ENDOTOXIN

	Animal no.	Blood pressure before endotoxin injection (mm. Hg)	Blood pressure before sacrifice, 24 hr. after endotoxin (mm. Hg)	Occlusive glomerular lesion *
Group IV	1 †	202		+++
DOCA hypertension + 1 mg. and	2	190		++
2 mg. of endotoxin, 12 hr.	3	175		++
apart	4	162		o
	5	164		o
	6 †	170		++
Group XI	1		198	++
DOCA hypertension + 400 μg. of	2		220	++
endotoxin once weekly for	3		202	+++
6 mo.	4		196	++
	5		222	+++
	6		180	++
	7		190	++
	8		182	+
Group XII	1		202	++
DOCA hypertension + endotoxin	2		156	o
tolerance + 400 μg. of endo-	3		215	+++
toxin once weekly for 6 mo.	4		190	++
	5		194	++
	6		190	o
	7		158	o
	8		148	o
	9		186	+
	10		192	++

* Severity of lesions is graded by number of plus signs; o indicates absence of lesion.
† Died 10 hours after first injection of endotoxin.

Long-Term Experiments with Endotoxin

Groups VI, VII, VIII and IX. Since the histologic appearance of the organs in all these groups was essentially normal, it is unnecessary to describe each group in detail. Suffice it to say that the substitution of 1 per cent NaCl for water did not produce a significant rise in blood pressure and did not predispose the animals toward the development of renal lesions during the long-term treatment with endotoxin.

Group X. The pathologic features previously outlined as characteristic of DOCA hypertension were severe (hypertension of 6 months duration) and quite similar to those found in malignant nephrosclerosis (Figs. 3 and 4). Sections of kidney showed marked fibrous thickening of small arteries and arterioles with some concomitant narrowing of the lumens, subintimal fibrinoid degeneration of arteriolar walls, fibrous thickening of glomerular basement membranes and, occasionally, complete fibrous obliteration of glomerular tufts. There were varying degrees of tubular damage ranging from dilatation and hyaline cast formation to atrophy with the appearance of minute rings of cells.

Examination of the heart, liver and spleen showed no noticeable pathologic alterations.

Groups XI and XII. The two groups are considered together since "endotoxin tolerance" did not influence the development of the occlusive lesions and did not prevent the appearance of DOCA hypertension. The weekly administration of 400 μg. of endotoxin did not significantly alter the course of DOCA hypertensive disease. Systolic blood pressures reached hypertensive levels approximately 3 weeks after the implantation of DOCA pellets and remained at this high level throughout the experiment. The average weight of these animals was lower at the end of the experiment than that of uninephrectomized controls receiving endotoxin.

Microscopic examination of the kidneys revealed a striking homogeneous, intraluminal deposit, resulting in occlusion of glomerular vessels, afferent arterioles and small arteries; these changes were similar to those found in group IV. In addition, the pathologic features due to long-standing DOCA hypertension (see group X) were also present.

Small, scattered focal collections of chronic inflammatory cells were found in the renal cortex in most of these rats, but they lay in relation to vascular scars and were not the result of any infectious process. The sub-epithelial tissues of the renal calyces were free of any inflammatory cells in all but 3 rats. The azan-carmine stain was used to distinguish the generalized basement membrane thickening and fibrosis from the intraluminal deposit of fibrinoid which occluded glomerular vessels. The latter stained bright red; the fibrous tissue thickening the glomerular basement membranes and the walls of small arteries and arterioles stained blue.

Review of the lesions disclosed that the most marked deposition of fibrinoid occurred in animals having the highest blood pressure recordings (Table II). Four of 18 rats failed to develop this lesion.

Three animals showed small focal areas of myocardial fibrosis in the

left ventricle. Examination of livers and spleens revealed no significant pathologic alterations.

Discussion

DOCA and other sodium-retaining adrenal cortical steroids are believed to play a role in the pathogenesis of hypertension. The substitution of 1.75 per cent NaCl for water may, in itself, cause hypertension in adrenalectomized rats.[12] The long-term administration of DOCA and salt together in experimental animals seem to act synergistically to produce a hypertensive syndrome with concomitant renal alterations and to sensitize such animals to vasoactive agents.

Renin injections to early DOCA-hypertensive animals elicited degenerative changes in glomeruli which bore a resemblance to renal lesions found in malignant nephrosclerosis. Attention has been brought to the fact that the existence of DOCA hypertensive disease is a prerequisite to the syndrome elicited by renin since the concurrent injections of DOCA and renin do not produce glomerular lesions.[11]

The renal cortical necrosis reported by Fiore-Donati and Erspamer[4] and Waugh and Pearl,[13] and the thrombotic lesions observed by Page and Glendening[6] following 5HT administration were not observed in the present study. It should be pointed out, however, that the doses and route of administration of 5HT by these investigators were quite different from ours.

The foregoing observations afforded definite evidence that the intraperitoneal administration of endotoxin to rats with early DOCA hypertension produced a characteristic glomerular lesion reminiscent of some of the lesions found in eclampsia and malignant nephrosclerosis. The findings seemed to indicate that DOCA hypertensive disease was prerequisite to the appearance of endotoxin-induced renal lesions in the rat since in the absence of DOCA hypertension, neither endotoxin nor the substitution of 1 per cent NaCl for drinking water brought about lesions in the kidney. Some of the degenerative glomerular changes elicited by renin or long-standing DOCA hypertension were found to a lesser extent after endotoxin administration to rats with early DOCA hypertension. In the latter case, however, the presence of intracapillary fibrinoid thrombi was the most impressive observation.

The rat generally is quite resistant to the effects of bacterial endotoxin, while man, the rabbit and the dog are much more susceptible. In the rabbit, for example, two intravenous injections, spaced 24 hours apart, produce the familiar generalized Shwartzman reaction, characterized by the intracapillary deposition of fibrinoid material in glomeruli and by bilateral renal cortical necrosis.[7] Gronvall and Brunson[14] pro-

duced this phenomenon in rats by the intraperitoneal administration of endotoxin in conjunction with the high molecular weight acidic polymer, sodium polyanethanol sulfonate (Liquoid). The lesions produced by the injection of endotoxin to DOCA-hypertensive animals described in this paper have a resemblance to the renal vascular lesions seen in the generalized Shwartzman reaction, in that the intraluminal deposit of fibrinoid was a prominent finding in both cases.

It is important to emphasize that there was a direct relation between the severity of the occlusive phenomena and the height of blood pressure in our experiments. Animals that failed to develop these lesions were generally those with near normal blood pressures. The indication is that high blood pressure alone or increased circulating DOCA with concomitant sodium retention, or both, can "prepare" the glomerular vessels for the "provocative" effects of endotoxin as does the "preparatory" dose of endotoxin in the production of the generalized Shwartzman reaction. In the same manner pregnancy seems to be able to take the place of the "preparatory" dose of endotoxin since in the pregnant rabbit a single injection of toxin will induce the renal lesions.[15]

Opinions differ as to the pathogenetic interpretation of renal lesions in human eclampsia. Recent publications by McKay and his associates [16,17] indicated that eclampsia was primarily a disorder characterized by intravascular thrombosis brought about by a mechanism similar to that of the generalized Shwartzman reaction. They postulated that pregnancy "prepared" the blood vessels for the provoking action of toxin which could originate either in the placenta or from a bacterial infection. The reduced fibrinolytic activity and the fibrinogenemia during pregnancy,[18] together with the increase in fibrinogen due to one endotoxin injection,[19] may facilitate the development of thrombosis. The reproduction of the renal features of human eclampsia in the experiments reported in this paper provide indirect evidence for the participation of endotoxin (or bacterial infection) in the pathogenesis of the disease. Mention should be made of a recent publication by Stamler [20] in which it was reported that pregnant rats kept on vitamin E deficient diet containing polyunsaturated fatty acids manifested the physiologic and histologic changes seen in eclampsia.

The observations in the present work have additional interest in that animals with early DOCA hypertension treated with endotoxin showed some degenerative changes in the glomerular basement membranes resembling those seen in malignant nephrosclerosis. It is thus conceivable that the human counterpart to this syndrome may be induced by bacterial infection, especially since it has been shown that hypertensive humans [21] and animals [22,23] are much more prone to succumb to pyelone-

phritis than normotensive subjects, and that a considerable proportion of patients with pyelonephritis develop malignant nephrosclerosis.[24]

Summary

Rats with DOCA hypertensive disease of short duration were subjected to various regimes of injections with renin, 5-hydroxytryptamine, and endotoxin. Rats with prolonged DOCA hypertension were also investigated with respect to long-term administration of endotoxin.

Four of 6 rats given renin exhibited an eclampsia-like syndrome accompanied by a degenerative glomerular lesion. 5HT treatment did not produce any noticeable pathologic alterations.

Endotoxin administered in various dose schedules to rats with early as well as long-standing DOCA hypertension produced a striking occlusive glomerular lesion associated with fibrinoid degeneration of glomerular basement membranes in 18 of 24 animals. The histologic lesion seen in these kidneys had the salient features of human eclampsia and reproduced some of the changes seen in the generalized Shwartzman phenomenon. Severity of renal involvement was related to the height of blood pressure.

It is suggested that DOCA hypertension as well as pregnancy may manifest increased renal vascular reactivity to endotoxin, resulting in the development of renal lesions.

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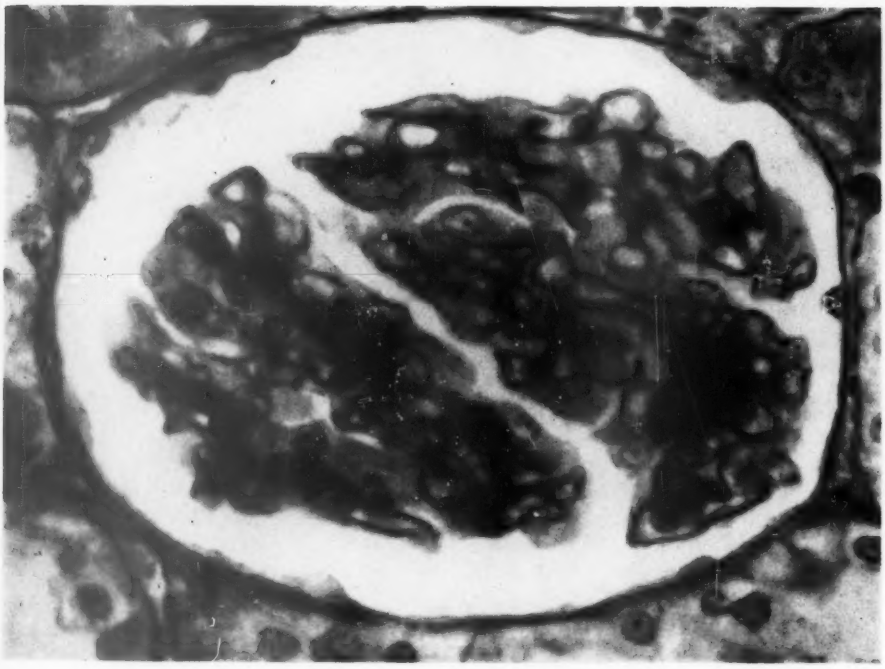
[*Illustrations follow*]

Legends for Figures

Illustrations were prepared from sections stained by the periodic acid-Schiff method.

Fig. 1. Normal glomerulus. × 600.

Fig. 2. Glomerular tufts in a rat with DOCA hypertension of short duration, showing minimal basement membrane thickening. × 800.



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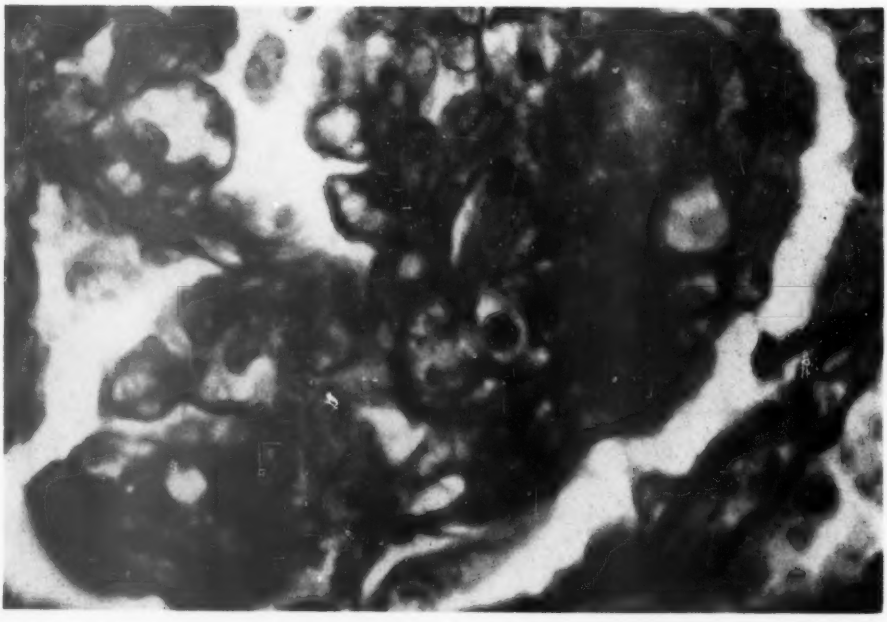

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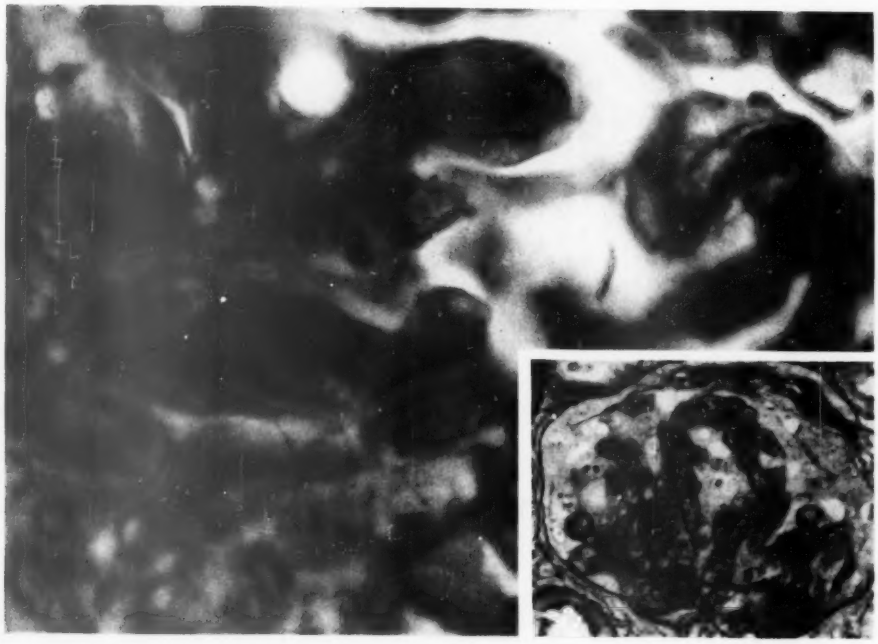
FIG. 3. Glomerulus in a rat with DOCA hypertension of long duration, demonstrating adhesions and fibrinoid degeneration of glomerular tufts. × 200.

FIG. 4. Higher magnification of a glomerulus in a rat with DOCA hypertension of long duration. Fibrinoid degeneration of tufts and adhesions are seen. × 800.

FIG. 5. Glomerulus in a rat with early DOCA hypertension given renin. Fibrinoid degeneration of the capillary walls and basement membrane is demonstrated. Occasional intraluminal PAS-positive deposits are seen focally. × 600.



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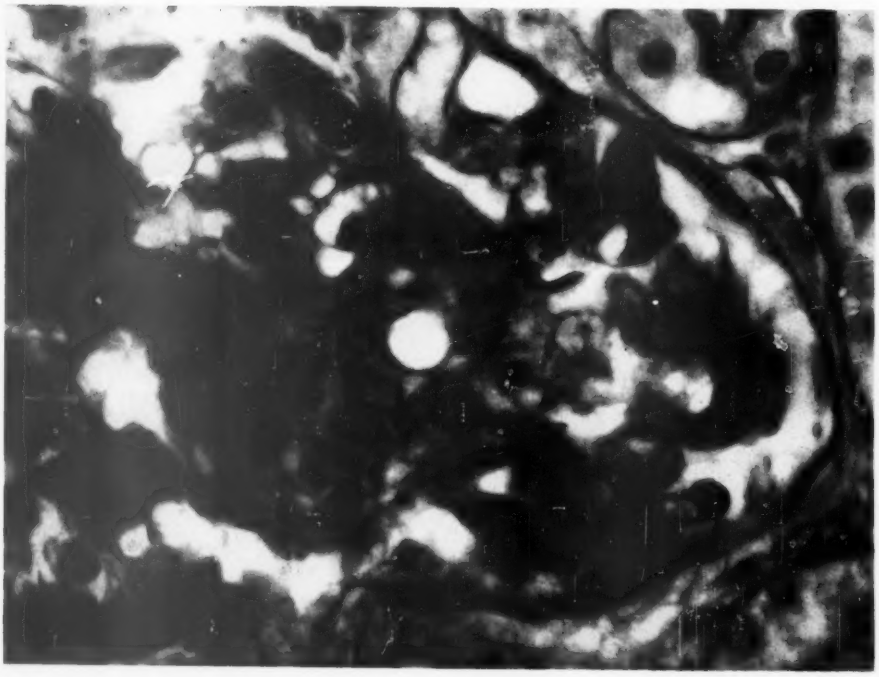

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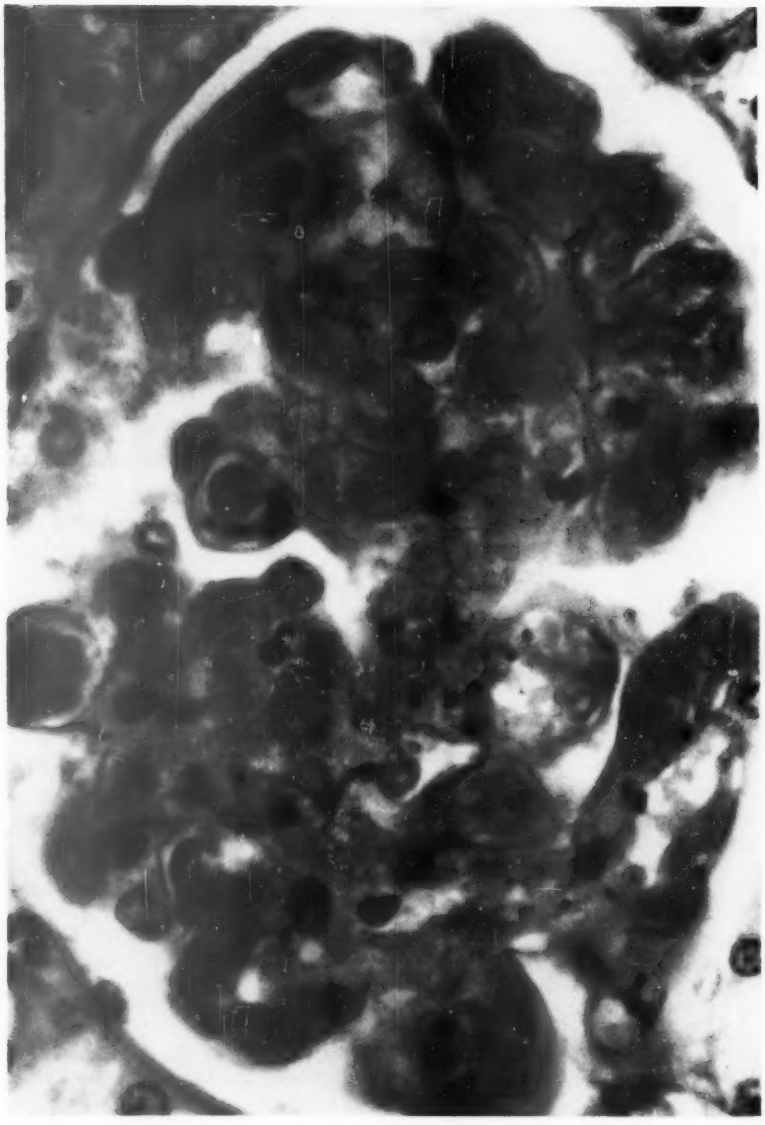
FIG. 6. Glomerulus in a rat with DOCA hypertension of short duration, given endotoxin. Note the striking deposition of intraluminal PAS-positive substance occluding glomerular capillaries. × 600.



6

THE HORMONAL INDUCTION OF A VAGINAL LEUKOCYTIC EXUDATE IN THE GERM-FREE MOUSE

DAVID L. BEAVER, M.D.

From the Laboratory of Pathology and Histochemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

It is a well known medical fact that the human vagina in the cornified state is more resistant to infection than when noncornified. For this reason, even today, estrogen therapy is frequently used in the treatment of infantile and senile vaginitis.

An equally well known fact in experimental pathology is the periodic recurrence of a vaginal leukocytic exudate during the estrous cycle of certain mammals, e.g., the rat, mouse, and guinea pig. This leukocytic infiltration is seen with such regularity and constancy that it has for years been accepted as a natural part of the cycle. It occurs during metestrus-2 and diestrus[1] (stages IV and V of Long and Evans[2]), at a time when the vagina is in the uncornified state and is possibly susceptible to secondary infection by normal, potentially pathogenic, vaginal flora. It, therefore, became of interest to ascertain whether or not an acute inflammatory cellular exudate of this type could be produced in the germ-free animal.

METHOD

A total of 7 white mice, divided into 2 groups, were used for the experiment. Each group of animals was born and reared in separate standard Reyniers' holding tanks under accepted germ-free conditions—i.e., by common cultural methods they were free of bacteria and fungi.[3-6] The 3 mice in the first group had been used previously for breeding and had given birth to several litters; the second group consisted of 4 mature young adults, which 3 weeks prior to injection had been transferred to a Trexler type plastic tank.[6] Material for injection was contained in 1 cc. sterile glass ampules and was taken into the tanks via a chemical lock (aerosol fog of 2 per cent peracetic acid with detergent).

Each animal of the first group received 5 mg. of progesterone (Eli Lilly and Company) in 1 cc. of sesame oil given subcutaneously in 3 divided daily doses, the animals being sacrificed on the fourth day, 24 hours after the last injection. In the second group each animal received the 5 mg. of progesterone in a single 1 cc. subcutaneous injection and was sacrificed 48 hours thereafter.

The mice, along with the usual material for culture (excreta, food, water, tank debris, and a test ampule of progesterone), were removed from the tank and the animals were immediately sacrificed. Cultures were also taken from each vaginal tract, which, with the entire genital system, was then fixed in 10 per cent buffered neutral formalin. Subsequently the tissues were embedded in paraffin and stained

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with azure A-eosin B. Additional sections of the vaginal tracts were stained by the periodic acid-Schiff method, methenamine silver, the Gram method and the Brown-Brenn technique.

Results

All animals showed an extensive neutrophil infiltration of the vaginal mucosa as well as an exudation of cells in the vaginal lumen (Fig. 1). The greatest collection of leukocytes was in the stratum spinosum (stratum germinativum, polygonal cells[1]) just beneath the surface cellular layers. Intra-epithelial micro-abscesses were common (Figs. 1 and 2). In addition, there were focal degenerative changes in individual cells of the prickle cell layer and upper stratum germinativum; these consisted of shrunken eosinophilic hyalinized cells with pericellular halos. Many of these hyaline masses were also surrounded by leukocytes (Fig. 2). In some areas the infiltration was almost confluent and involved the upper two thirds of the epithelium; in other areas within the underlying stroma many neutrophils were just beginning their emigration toward the surface. In the vaginal lumen, desquamated nucleated epithelial cells as well as keratinized forms were admixed with the leukocytes. No identifiable organisms were seen in appropriately stained sections, and all cultures were negative.

Discussion

It is technically somewhat difficult to follow the estrous cycle in the germ-free mouse, for the animal must be held and the smears made while generally wearing 3 pairs of gloves. The element of trauma, therefore, with resulting vaginal cornification[7] or pseudopregnancy[8] remains a constant possible source of error. Moreover, the scarcity of germ-free animals makes wholesale sacrifice of large numbers, in an effort to catch the animals in various phases of the cycle, impractical at the present time. Thus, although the selection of artificial hormonal stimulation was a compromise, it nevertheless afforded a relatively infallible method of producing the exudative phase independent of the natural cycle.[9]

The results of the experiment indicate that hormonal stimulation alone, in the absence of bacteria, is quite capable of producing a leukocytic response. The mechanism is not clear. The acute inflammatory cellular exudate could be secondary to the epithelial degeneration and necrosis. On the other hand, the epithelial destruction could be brought about by the enzymes of the accumulated white cells. The former process is a well recognized pathologic reaction; the latter is not and would, in addition, require an explanation for the leukotaxis to this particular location. The detached cornified layer cannot be implicated, for even

when in direct contact with viable responsive tissue, keratin usually evokes a chronic inflammatory and foreign body reaction, rather than a neutrophil exudate. Regardless of pathogenesis, however, the response is initiated by progesterone and probably results from a specific localized hormonal effect on the vaginal mucosa. The subsequent neutrophil infiltration is due to a considerably more complex phenomenon than to the simple loss of a protective cornified surface.

Although the tissue reaction described above is similar to that seen during a normal cycle in the non-germ-free state,[10] it cannot be concluded on the basis of the present experiment that the estrous cycle in the germ-free mouse necessarily parallels that of the conventional animal. Persistence of the leukocytic infiltration, rate of removal of desquamated cells, keratinolysis, and fluctuations in pH [11] are certainly factors which might influence the length and appearance of the various stages of the cycle. Such a comparison, therefore, would be entirely speculative and unjustified.

Summary

Since the noncornified vagina is susceptible to infection, and since the leukocytic infiltration in the murine estrous cycle occurs during the noncornified state, it became of interest to determine whether this cellular exudate was secondary to the normal, potentially pathogenic, vaginal flora or whether it could be produced by hormonal stimulation alone. Using the germ-free mouse and artificial hormonal stimulation, it was found that progesterone, in the absence of bacteria, was quite capable of producing a typical inflammatory response.

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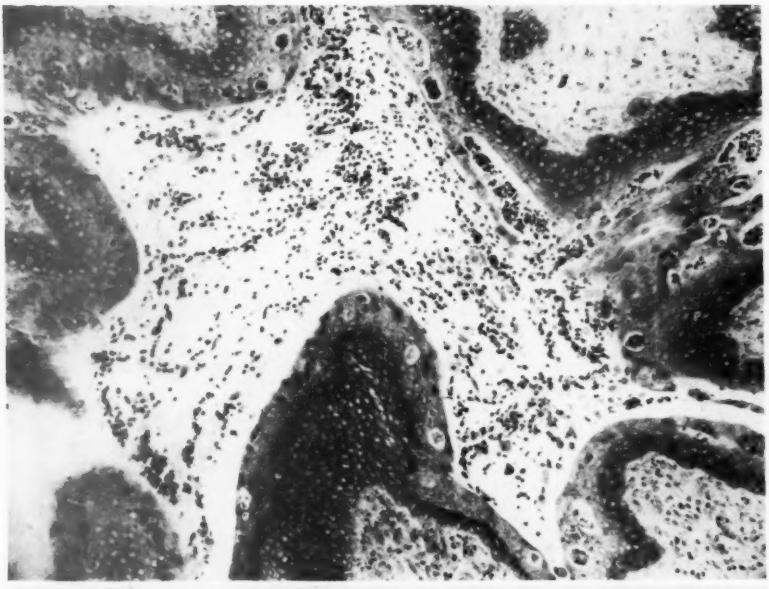
LEGENDS FOR FIGURES

FIG. 1. Germ-free mouse. Cross section of hormonally stimulated vaginal tract, showing extensive leukocytic infiltration. Azure A-eosin B stain. × 117.

FIG. 2. Sterile vaginal mucosa 48 hours after the administration of progesterone. Note focal intra-epithelial accumulations of leukocytes, many surrounding shrunken hyalinized necrotic cells of the stratum spinosum. Azure A-eosin B stain. × 210.



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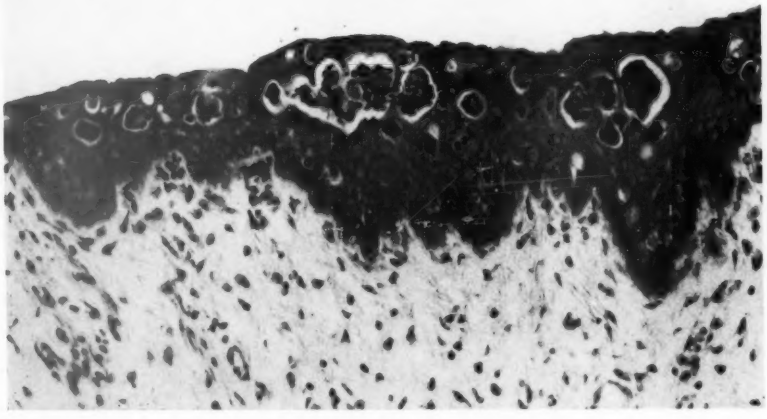

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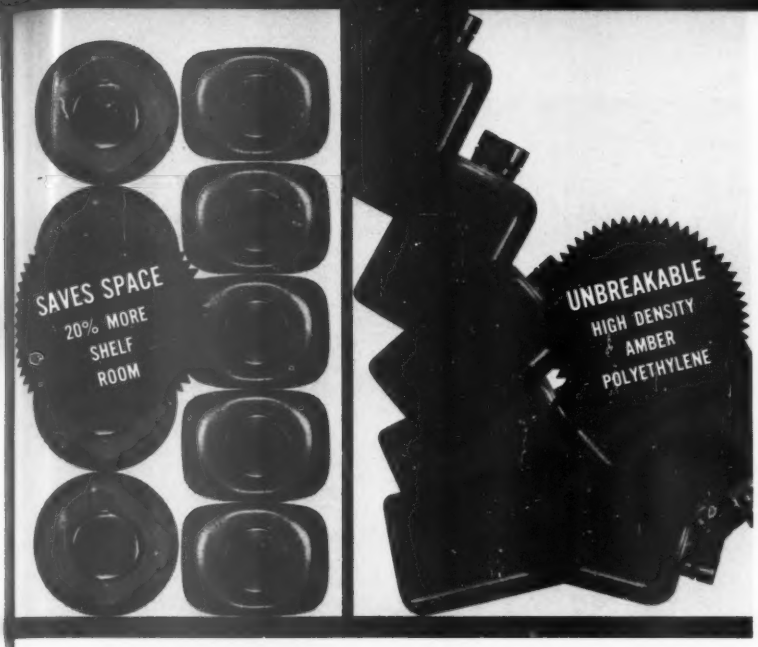

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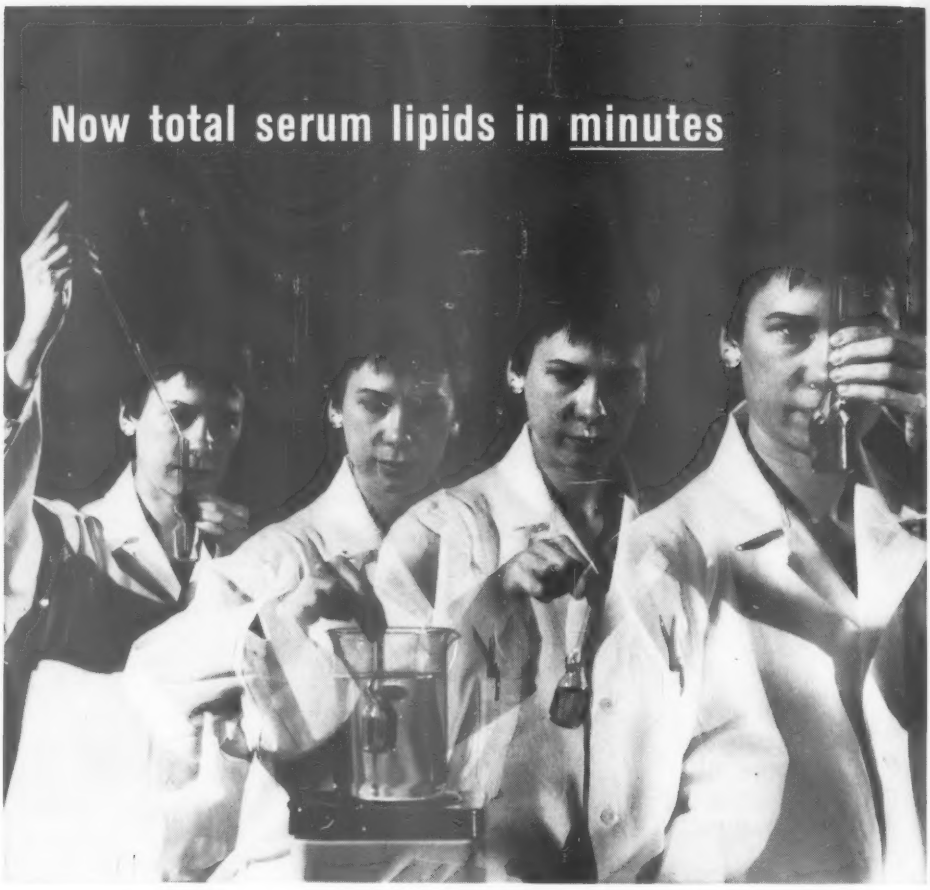

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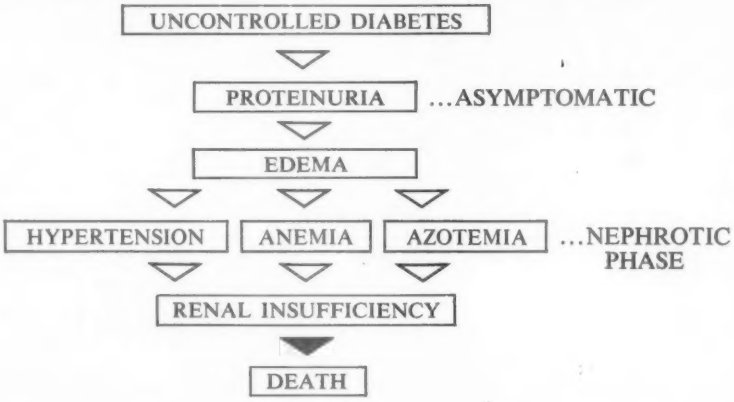
NATURAL HISTORY OF DIABETIC NEPHROPATHY



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